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A study of the nitrogen content of dune soils, with particular reference to the effects produced by the sea-buckthorn, *Hippophae rhamnoides* L.

## Thesis

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Submission for the degree Bachelor of Philosophy  
in the discipline of Chemistry  
in the Open University

A study of the nitrogen content of dune soils,  
with particular reference to the effects produced  
by the sea-buckthorn, *Hippophaë rhamnoides* L.

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A study of the nitrogen content of dune soils,  
with particular reference to the effects  
produced by sea-buckthorn, *Hippophaë rhamnoides* L.

Alan L.F. Mason (Ref No

HDK 1331)

### Summary of thesis

The history of research into the chemistry of dune soils is reviewed, with particular reference to the presence of nitrogen compounds, and also the effects created by the nitrogen-fixing shrub, sea-buckthorn (*Hippophaë rhamnoides*, L.).

Problems associated with the colonization of sand dunes by *Hippophaë* were investigated in the main study, involving soil sampling at selected sites between December 1980 and November 1981 on the Norfolk coast near Hunstanton. *Hippophaë* scrub of different ages was chosen, and control sites were left undisturbed. At experimental sites thick polythene sheeting was buried below the surface sand to prevent the downward percolation of solubles.

Subsidiary investigations were made of other coastal sites in Britain, some with, and some without, *Hippophaë* colonies. The soil samples were analysed principally for nitrate ion, but also for ammonium, phosphate, and carbonate ions.

The investigations revealed that nitrate levels in dune soil are proportional to the age of the *Hippophaë* scrub. A seasonal variation in nitrate levels was observed, with peaks in April and September. A model nitrogen economy was proposed to account for this.



Statistical tests revealed no significant differences between control and experimental sites suggesting that nitrate is derived principally from Hippophaë rather than the leaf litter or animal products, probably by routine disintegration of the root nodules.

The subsidiary sites lacking Hippophaë colonies had no detectable nitrate, or very low levels ( $< 0.5$  ppm) even when heather scrub (Studland Heath, Dorset) or hawthorn scrub (Daymer Bay, Cornwall) was present. This confirms the view that the nitrate detected at Hunstanton came from the Hippophaë scrub.

Where Hippophaë was prolific (Gibraltar Point, Lincolnshire) the levels of nitrate detected were higher than at Hunstanton, even in the uncolonized sand of the beach, due possibly to the circulation of nitrates within the ground water of the dune system.

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## Notes

### 1 References

Each reference in the text is given by the surname(s) of the author(s) and the year of publication within brackets.

### 2 Bibliography

Many of the references occur more than once in the text, so there is a single bibliography giving the authors in alphabetical order at the end of the thesis.

### 3 Figures, Maps, Tables and Graphs

The various figures, maps, tables, and graphs included in the text have been numbered sequentially within each chapter so that Graph 3.11 indicates the eleventh graph in Chapter 3.

### 4 Numbering of parts of the thesis

The first number indicates the chapter, the second is the section, and the third a subsection.

# List of Tables, Graphs, Figures and Maps

Each of these is numbered sequentially within each chapter.

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## Introduction

This study arose from a long-term enthusiasm for the ecology of sand dunes in general, and interest in sea buckthorn in particular.

Dunes provide an interesting habitat for introducing students to some of the general principles of ecology. The variations observed in the frequency of species within a single dune system help to give some idea of the problems of heterogeneity of distribution patterns and the validity of sampling methods. In trying to examine climatic and edaphic factors it is quickly evident that a short study can do no more than demonstrate methods. A full understanding demands a daily and seasonal monitoring of these factors.

Sea buckthorn is a handsome and readily identifiable plant. Its distribution is local and irregular, so that it is found in embarrassingly invasive profusion in some areas, but is totally absent in neighbouring regions which are seemingly identical. It does not always take successfully when artificial planting is attempted. There are few woody plants in Britain capable of colonizing bare, shifting sand dunes, as the buckthorn does. It is a big, tough shrub for such an unpromising soil medium as bare sand. The explanation seems to lie in its ability to fix atmospheric dinitrogen by means of its root nodules. Once it is successfully established a whole range of other plants begin to carpet the sand around it.

Given the opportunity to carry out a year-long study I decided to look more closely at the effects of buckthorn colonization on dune soils. There were a number of simple

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questions which, as far as I know, had not been investigated on a seasonal basis.

Primarily, is it possible to measure the level of soluble nitrogen compounds in dune soils? Do these levels vary during the course of a year? Does the sea buckthorn increase the nitrogen level of dune soil in its immediate vicinity? Is there a relationship between the age of buckthorn scrub, and soil nitrogen levels? Is it possible to distinguish between the effects of normal litter breakdown, and possible effects of root nodules, in increasing soil nitrogen levels?

Once the pilot study had answered the first, crucial question, it was possible to embark upon an experimental design to find answers to some of the other questions. Preliminary examination of the literature indicated that although ecological studies of buckthorn had been made in the field, and physiological studies had been made in the laboratory, there were few on the effects of buckthorn on dune soil, in the field situation. Some of the studies on dune soil chemistry seemed to indicate that an examination of nitrogen in particular would be worthwhile. The collation and comparison of the results of a variety of workers would provide a useful yardstick with which to judge the results obtained in my own study.

Attention has been drawn to both the nitrogen economy of dunes, and also the nature of the scrub communities.

"Ecologists have tended to neglect the study of nitrogen in natural communities ... leguminous plants ... exist on poor dry soils of little agricultural worth and although their presence must influence the nitrogen economy of such sites,

the effects have not been much investigated by ecologists."  
(BANNISTER 1978 pp. 182-3)

"In sand dunes, which are recent deposits, and low in nitrogen plants such as the non-leguminous sea-buckthorn have a symbiotic association which results in increased plant and soil nitrogen in stands of increasing age." (BANNISTER 1978 p. 183).

It has been stated that "the study of the ecology of dune scrub is in its infancy" (RANWELL 1972 p. 181) and the author has strongly encouraged a trend towards the "detailed study of the unit 'fragments' of the ecosystem" such as Hippophaë scrub" (p.181).

1. Chapter One: A review of the study of nitrogen in  
dune soils

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The direct measurement of nitrogen in dune soils was a late development, but many of the earliest systematic studies were concerned with the presence of organic matter. As this has a bearing on the level of soluble nitrogen compounds available to colonizing plants, it is worth examining some of the findings in detail.

The importance of soil factors, and general climatic factors in plant distribution had been known for a long time, but towards the end of the nineteenth century, workers in Europe and North America made an attempt to define the relationship more clearly. In formalizing the classification of plant communities in Europe botanists like Warming, Schimper, Graebner, and Nilsson had tried to relate the distribution of these communities to simple factors like soil moisture and organic content.

It was soon appreciated that the relationship was far from simple. There was a historical component, as well as a contemporary one. Some communities were of long-established nature, others were more recent. The first formal proposal of a theory of succession was made by Henry Cowles, following his detailed studies of the plant communities in the area of Lake Michigan, U.S.A. (COWLES 1901).

He had spent several years in studying the dune communities around the margin of the lake (COWLES 1899). The presence of organic matter is very important to the succession process, and to the development of stable plant communities in such an impoverished soil. The theory of succession has subsequently proved to be a most valuable generalization, in its wide applicability to both plant and animal populations. It has

been possible to explain major changes in terms of a relatively small number of causal factors. Simple agents like water content, organic content, pH, or calcium carbonate content have been shown to be of particular importance. The great weakness of the early pioneering studies lay in their purely qualitative nature. The application of quantitative methods to plant distributions and edaphic factors came later.

In Britain a series of studies of dune communities was made following Cowles' important generalization. A range of coastal sites were examined, including Kenfig Burrows, Glamorgan (ORR 1912), Anglesey (WORTHAM 1913) Culbin Sands, Moray (PATTON and STEWART 1914) the Isle of Man (HARTLEY and WELDON 1914) the English west coast (WATSON 1918) and Berrow, Somerset (THOMPSON 1922).

This review is limited to a small number of papers having a direct bearing on the nitrogen-, and latterly the nitrate-content of dune soils. These are indicated in the following list

- 1.1 Early dune soil analyses - the effect of organic matter  
on various other factors  
SALISBURY 1922, 1925  
WILSON 1960
- 1.2 Nitrogen deficiency and plant growth in dune soils  
WILLIS et al 1959  
WILLIS and YEMM 1961
- 1.3 The liberation of soluble nitrogen compounds from  
organic matter in dune soils

GÉHU and GHESTEM 1965

BROWN 1958

- 1.4 Effect of nitrogen-fixing colonizers on nitrogen levels in tropical dune soils  
DOMMERGUES 1963
- 1.5 Sea-buckthorn and impoverished soils  
ROUSI 1971  
PEARSON and ROGERS 1962  
SKOGEN 1972  
SLOET VAN OLDRUITENBORGH 1976
- 1.6 Release of nitrogen from nodules of Hippophaë  
SKOGEN 1972
- 1.7 Variation in the development and decay of root nodules of Hippophaë  
OREMUS 1979  
STEWART and PEARSON 1967
- 1.8 The relationship between the distribution of the endophyte and root nodulation in Hippophaë.  
OREMUS 1980
- 1.9 Causal factors in the inhibition of root-nodulation in Hippophaë rhamnoides at mature successional stages  
OREMUS 1981
- 1.10 Seasonal variation in soil nitrogen levels  
DAVY and TAYLOR 1974
- 1.11 Nitrogen fixation associated with marram grass -  
Ammophila arenaria



HASSOUNA 1962

HASSOUNA and WAREING 1964

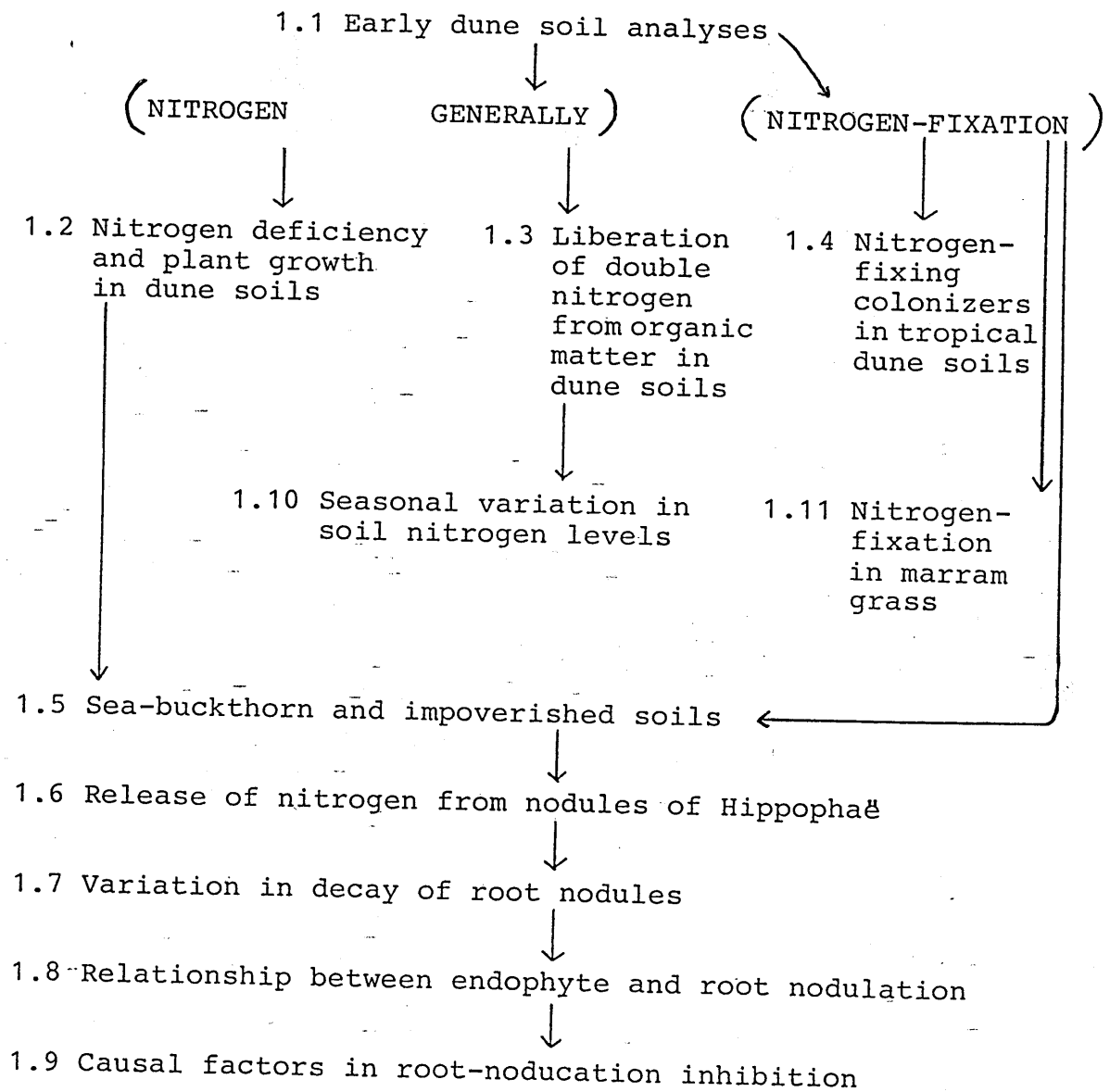
AHMAD and NECKELMANN 1978

ABDEL WAHAB 1975

ABDEL WAHAB and WAREING 1980

ABDEL WAHAB 1969

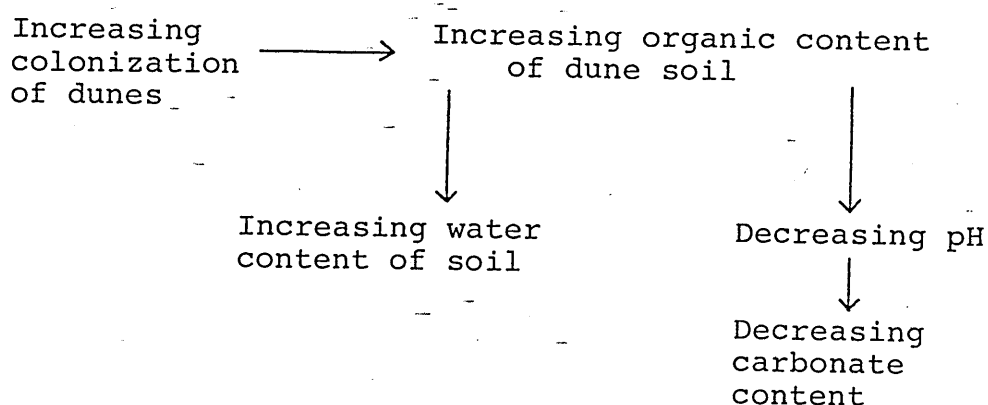
FIGURE 1.1 Relationship of the sections within Chapter One.



### 1.1 Early dune soil analyses; the effects of organic matter on various other factors.

The first comprehensive analysis of dune soils for a variety of edaphic factors was made at Blakeney Point, Norfolk (SALISBURY 1922). The study yielded much valuable data on the pH, water content, organic content, and carbonate content of the dunes. Chloride ion levels were recorded for a limited number of sites. Although no measurements of soil nitrogen were made it is worth examining Salisbury's paper in some detail because it bears upon the role of organic matter in dune soils. The great value of the work lies in the large number of samples collected and analysed. Although the statistical treatment of the results was rudimentary by modern standards the main conclusions emerged clearly. The various chains of causal relationships inherent in Salisbury's study can be summarized diagrammatically.

Figure 1.2 Trends in dune soil development



Some of these relationships emerged in the text of the paper and although the data support each of the relationships, the tables and graphs which were published do not always make this apparent. Fortunately all Salisbury's data were given

in a very full appendix, summarized here in Table 1.1.

The first relationship was described succinctly by Salisbury. "The dunes of successively greater age thus exhibit a perfect gradation in organic content, so that when more dune systems have been examined in detail it may be possible to determine their approximate age by this means" (p. 400). He went on to conclude that "the organic material is mainly responsible for the water capacity of dune soils (pp. 401-2).

Table 1.1 Analysis of dune soils, Blakeney, Norfolk  
(from Salisbury 1922)

DUNE REF. NAME	J & G Embryo	E Main ridge	D Lab. ridge	C * Ridge C	B Long Hills	A Hood
% O	0.36	0.50	0.53	0.86	1.15	2.69
% W		25.4			29.46	35.90
% C	0.425	0.341	0.155	-	0.010	0.010
pH	7.17	7.03	6.4	6.4	6.38	6.24

KEY DUNE REF. - in order of increasing age, A is the oldest dune

% O - average percentage organic content by weight  
(loss on ignition method - usually the mean of about a dozen analyses)

% W - "average percentage of water by weight"  
(mean of nine analyses)

% C - "average percentage of carbonate by weight"  
(mean of about a dozen analyses)

pH - (mean of over a dozen results)

\* At C the sample sizes were much smaller, only 2 for organic, and 5 for carbonate, so the level of confidence is much less than for the other dune sites.

The letters A to J represent different sample sites at Blakeney.

These two conclusions are demonstrated in Graph 1.1 derived from data in Table 1.1 Salisbury argued that if the increase in water content with the age of the dune is due largely to the increase in organic matter then a simple mathematical relationship should exist. The principal problem inherent in Salisbury's data was that he could not quantify the age of the dunes, which made it difficult for him to demonstrate his proposition mathematically. Consequently he made use of a simple ratio to eliminate age as a parameter. He argued that the ratio

$$\frac{\text{increase in water content}}{\text{increase in organic content}} \quad \text{is constant.}$$

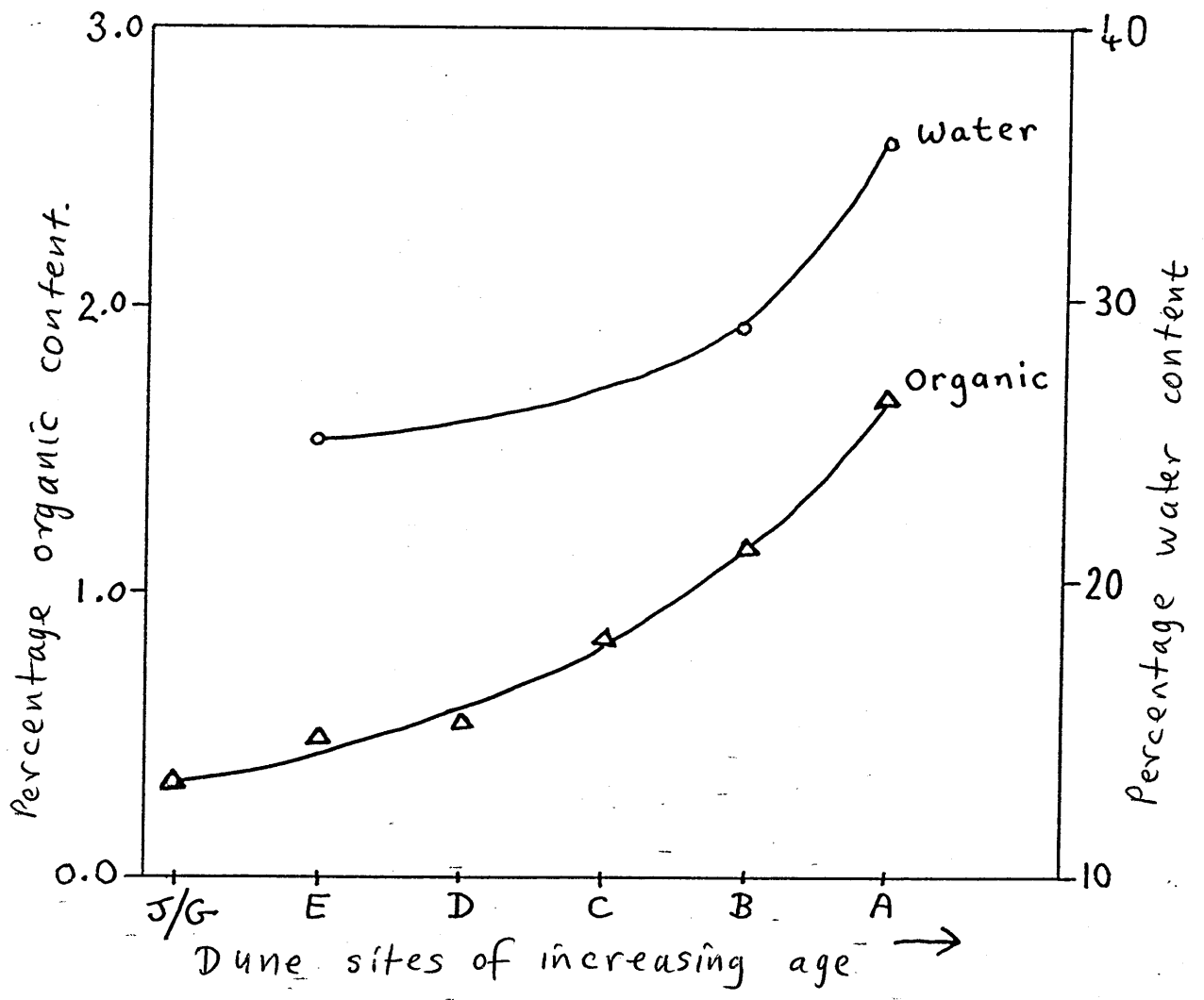
Because he estimated water content at only three sites, E, B and A this limited him to two ratios. He calculated

$$\frac{\% W_B - \% W_E}{\% O_B - \% O_E} \quad \text{and} \quad \frac{\% W_A - \% W_E}{\% O_A - \% O_E}$$

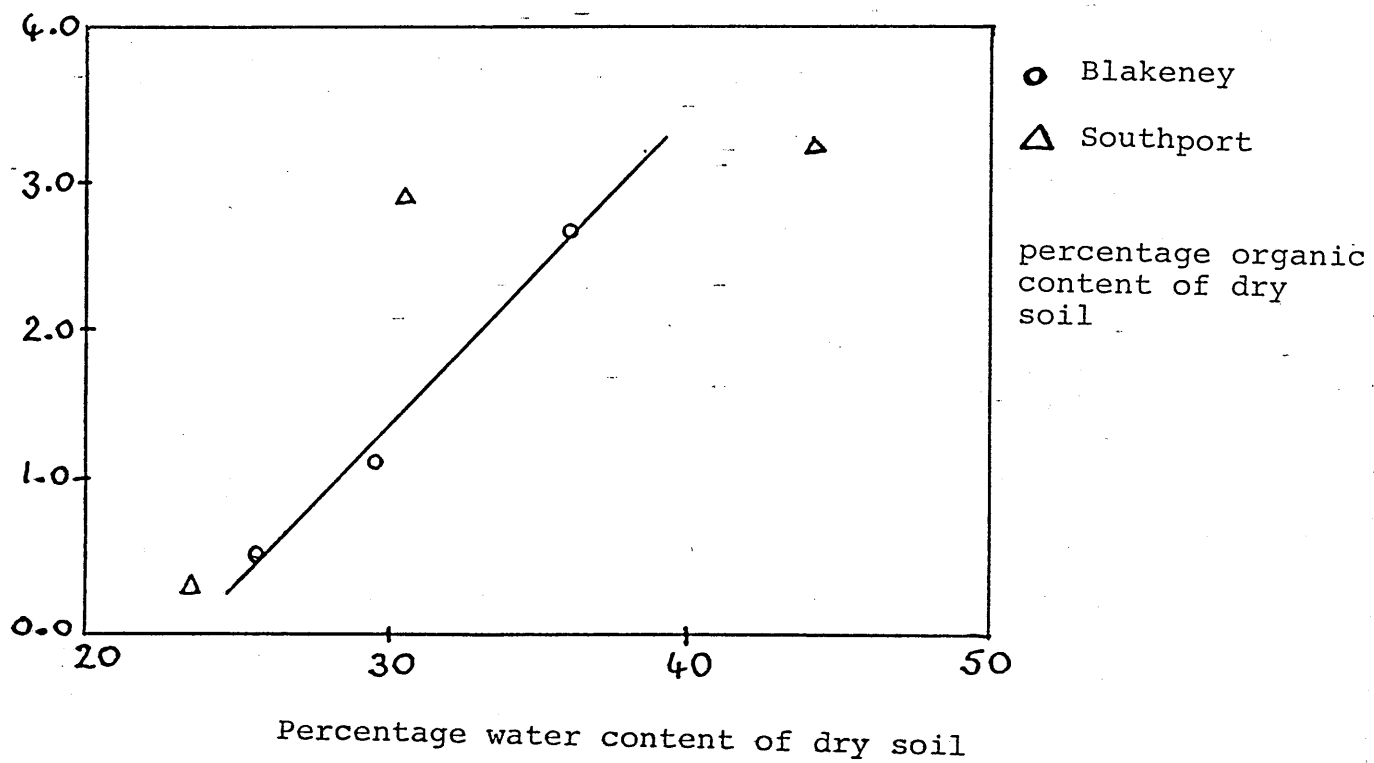
obtaining values of 4.68 and 4.79 respectively which seemed to be in good agreement. However there is an arithmetical error in the calculations. The values should be 6.2 and 4.79.

The validity of Salisbury's conclusions can be demonstrated more simply by plotting water content against organic content. (Graph 1.2) The data points lie in a straight line. When the data from Salisbury's Southport dunes study are included, it can be seen that there is considerable variation. A much larger number of samples would be required to establish the relationship more securely. It also indicates that the use of organic content as a method of dating was rather optimistic on the evidence available.

Graph 1.1 Association between water content, organic matter and dune age (data Salisbury 1922)

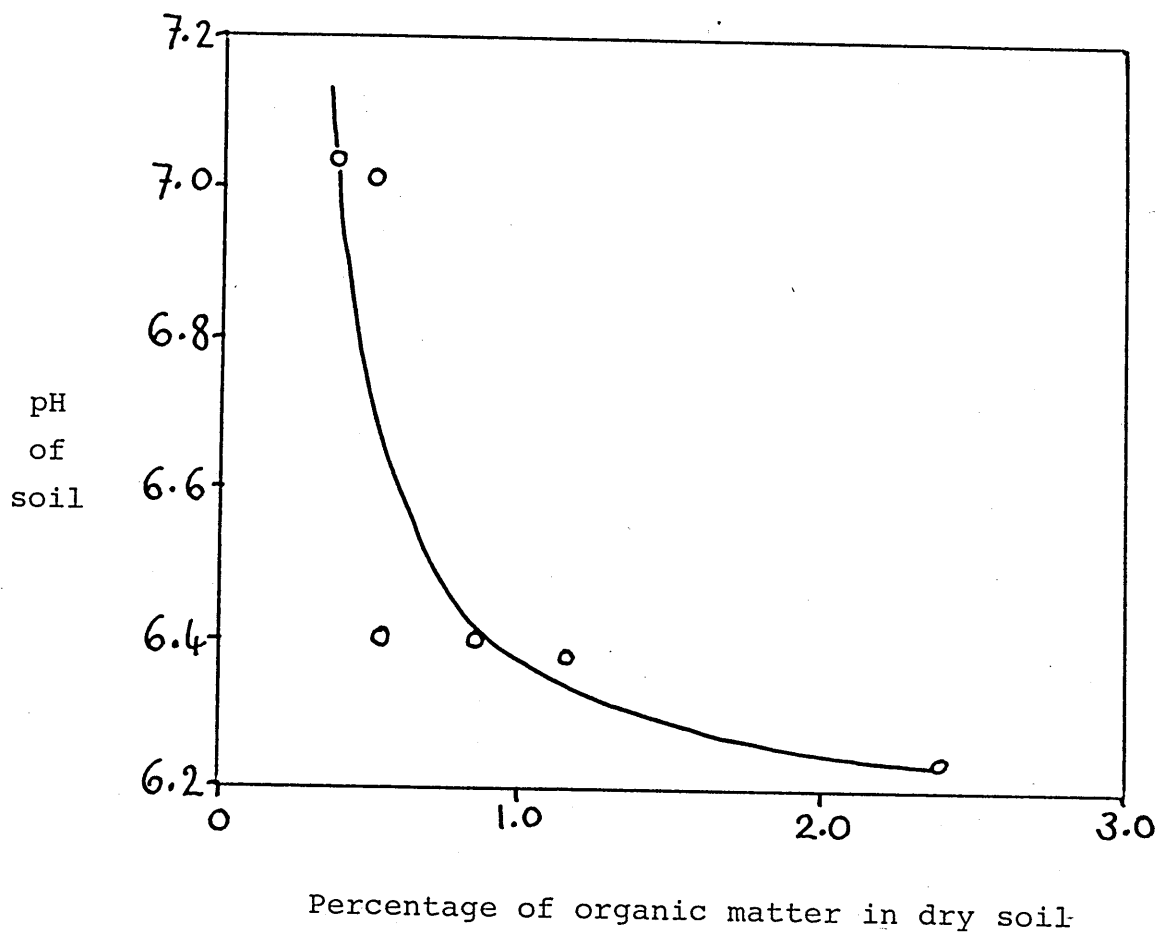


Graph 1.2 Association between water content and organic matter (Salisbury 1922, 1925)

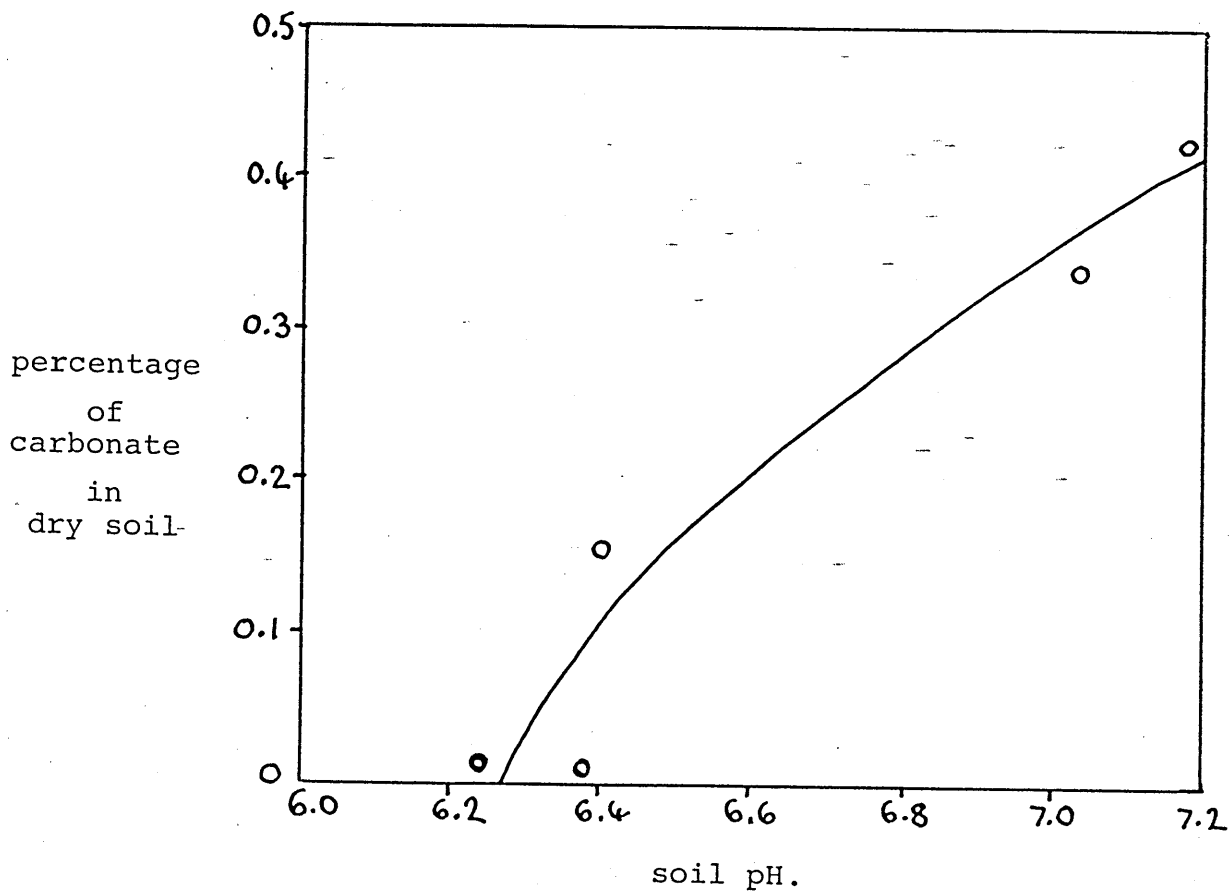


Graph 1.3 Decrease in soil pH with increase in content of organic matter (Salisbury 1922)

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Graph 1.4 Decrease in soil carbonate content with decrease in soil pH. (Salisbury 1922)



Salisbury commented on the decrease in pH value with the age of the dunes and he made indirect inferences about the role of organic matter and leaching in the corresponding decreases in carbonate content. The decomposition of organic matter in litter certainly yields organic acids, and it is the effect of these which dissolve carbonates. He showed graphically the reduction in the quantity of carbonate with increase in organic matter (p. 414).

The change in pH associated with increase in organic matter is shown in Graph 1.3 as a curvilinear relationship. The data on decrease in carbonate relative to pH are given in Graph 1.4.

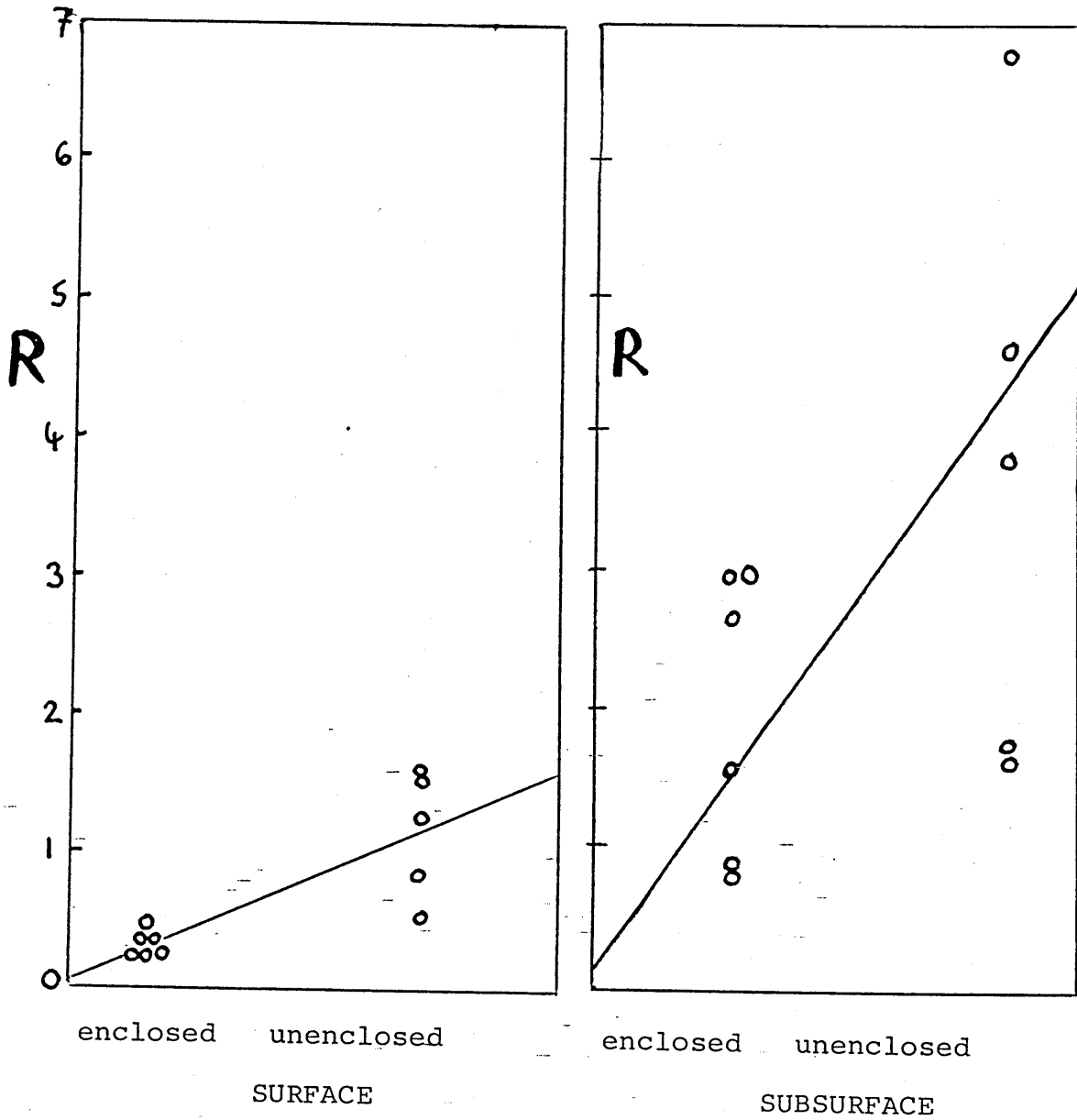
One of the most interesting aspects of Salisbury's paper was an experiment to determine how much organic matter was contributed to dune soils by rabbit droppings. Two areas were selected, and both unenclosed (control) sites, and enclosed (experimental) sites were prepared in each. The results indicated quite a high degree of variation but the overall picture was clear, as indicated in Graph 1.5 from data in Table 1.2.

Table 1.2 Contribution of rabbit-droppings to organic matter of dune soils. (Salisbury 1922)

	control (unenclosed)		Experimental (enclosed)	
	surface	subsurface	surface	subsurface
Area A	1600	3879	352	928
	1248	6784	352	3008
	-	-	480	2720
Area B	832	1824	288	1600
	512	4640	320	864
	1536	1568	288	3008

The data are for the number of droppings/square metre.

Graph 1.5 Contribution of rabbit droppings to the organic content of dune soil



$R$  = concentration of rabbit droppings per  $m^2 \times 10^3$



The number of droppings was always greater in the subsurface soil. This was due to burial by the accretion of sand, according to Salisbury. The enclosed plots were fenced off from rabbits for  $3\frac{1}{2}$  years. In general there were fewer droppings within the enclosed plots than the control plots. Salisbury estimated (p. 407) that the annual contribution from rabbit droppings was  $34 \text{ g}/312 \text{ cm}^2$  ( $1.09 \text{ Kg/m}^2$ ) or about 0.18 % by weight of the soil. There are no details of how the experimental plots were enclosed, but it would have been necessary to prevent dried droppings blowing into these sites from the outside. Salisbury alludes to "a clear tendency ... for the rabbit droppings to decrease in amount from the younger to the older dunes" (p. 407) due to the decreasing availability of preferred food plants.

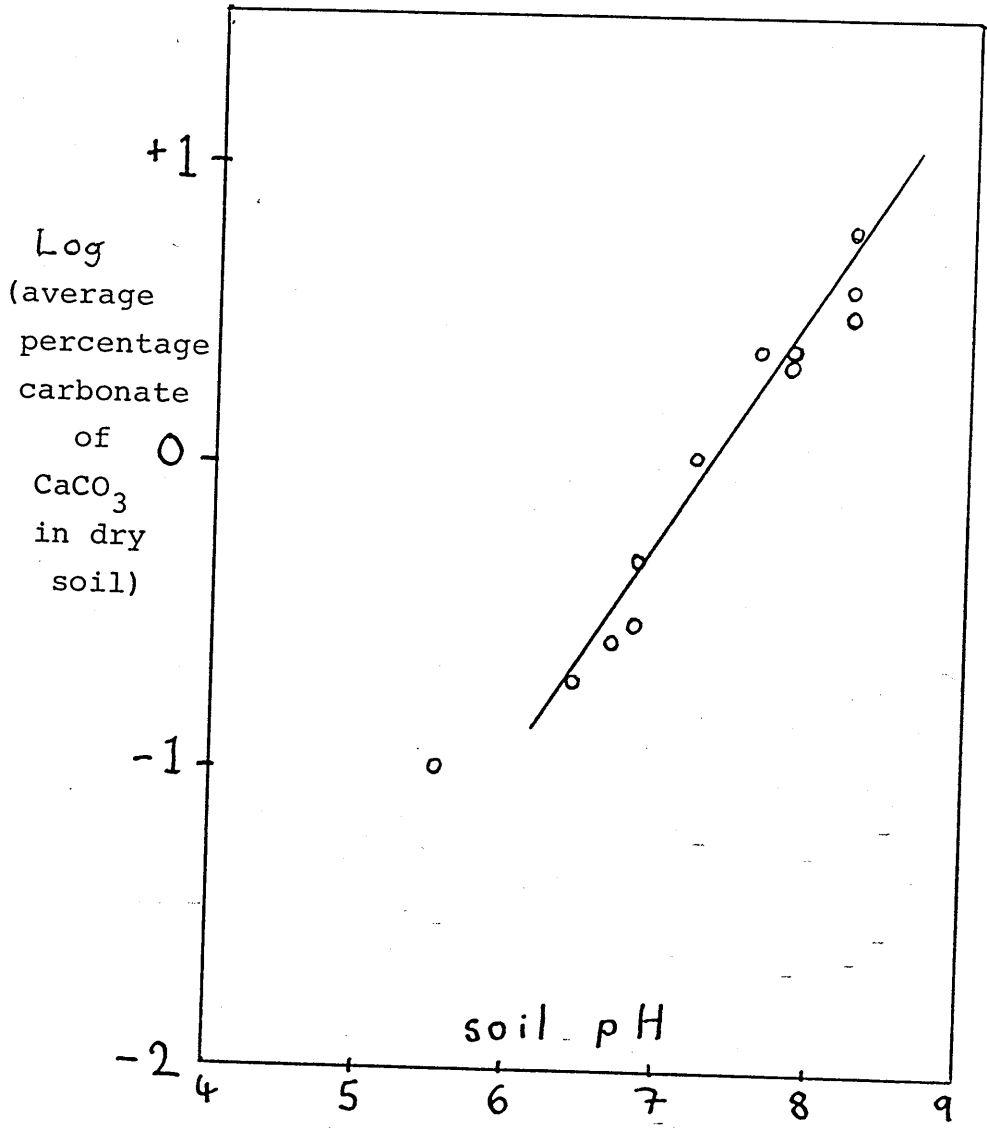
It was clear from these studies that any attempt to explain the developing quality of nitrogen-deficient dune soils would need to take into account the effects of animal wastes, particularly in the earlier stages of colonization.

The studies at Blakeney Point were followed up by further chemical analyses of the dune soils of Southport, Lancashire. (SALISBURY 1925) In this later paper, Salisbury referred to the difficulty of estimating the chronological age of the dune series at Blakeney, and he therefore chose the Southport area because it was possible to date the ridges by the use of old maps.

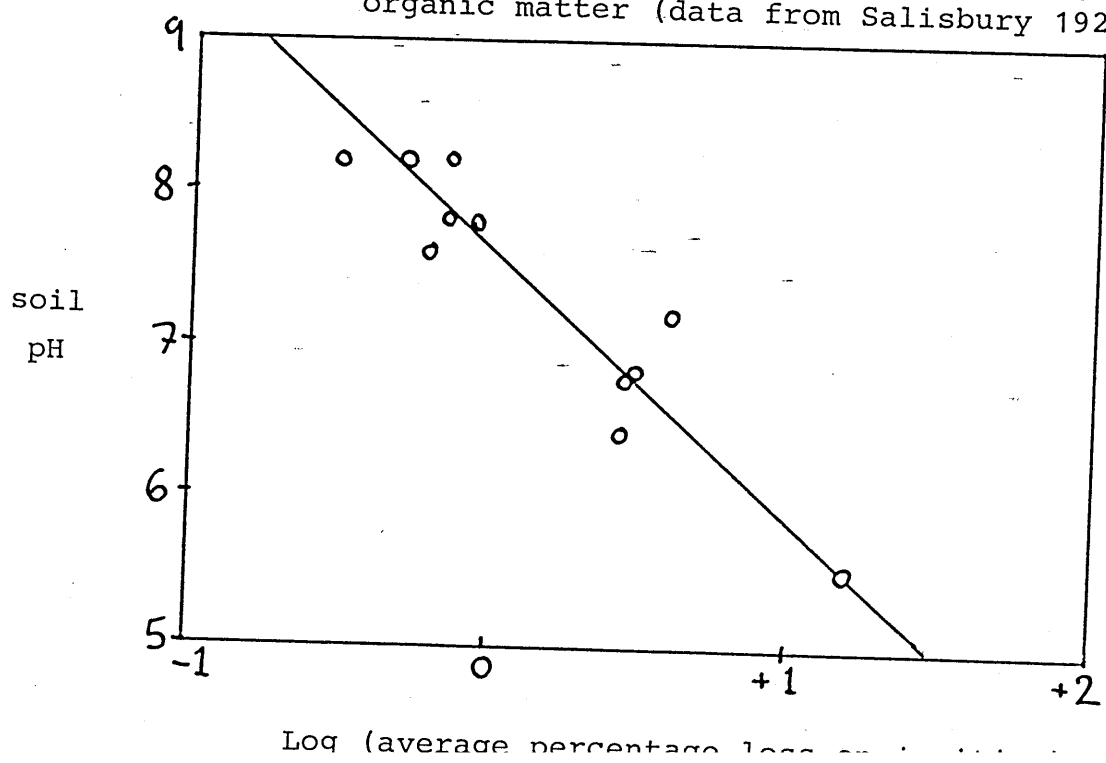
He was able to confirm the general conclusions obtained in his Blakeney study, and his data have proved altogether more satisfactory in establishing the mathematical relationships that he sought. He made some attempt to date the Blakeney dunes, in order to compare them with those at Southport. His



Graph 1.6    Decrease in soil carbonate content with increasing soil acidity (data from Salisbury 1925)



Graph 1.7    Increase in soil acidity with increase in soil organic matter (data from Salisbury 1925)



graph of increase in organic content with age of dunes is most instructive (p. 326). It reveals that the rate of accumulation of organic matter at the two locations is quite different. Consequently the content of organic matter in dune soils cannot be used to estimate the approximate age of the dunes, as Salisbury had hoped in his 1922 paper.

The data on decrease in pH with increase in organic matter give altogether more satisfactory results than those from Blakeney possibly because the ranges are much greater. Salisbury simply presented these data in a table, because he was much more interested in the problem of the leaching of carbonates. A log x log plot (i.e. pH against log organic matter) enables a regression line to be fitted quite satisfactorily to the data points (Graph 1.7).

Following upon Salisbury's interest in the leaching of carbonates a plot of pH against log carbonate content yields a most satisfactory set of data points for a regression line (Graph 1.6). It is likely that the much higher levels of carbonate in the dune soil at Southport assisted Salisbury in demonstrating this particular natural process. There is a certain irony in the fact that Salisbury, having gone to some trouble to date his dunes at Southport, could have ignored the actual ages in demonstrating a simple mathematical relationship between soil pH and soil carbonate level. The regression line yields an equation

$$y = Ax - B$$

where  $y$  = log average % carbonate content

$$x = \text{pH}$$

$$A = 0.804 \quad B = 5.834$$

This valuable demonstration of the basic chemical processes occurring in dune soils has been amply confirmed by a variety of studies made in subsequent years at widely different sites, in different parts of the world.

A detailed topographical study of the dunes of the Studland Peninsula, Dorset by means of historical maps enabled this series of ridges to be dated with considerable accuracy.

(DIVER 1933, DIVER and GOOD 1934, DIVER 1936). The dating of the dune system provided a valuable reference when a chemical analysis of the soils was made later (WILSON 1960) see Table 1.3.

Salisbury's data showed a contrast between dune systems poor in soil carbonates (eg Blakeney) and those rich in this respect (eg Southport). The Studland dunes are low in carbonates, and Wilson's study confirms the trends observed by Salisbury, in the relationship between organic soil content, and consequent pH. The high carbonate dunes showed a linear relationship between log % organic content and pH, while the low carbonate dunes showed a curved graph, where pH reaches a minimum value independent of further increases in organic matter (Graphs 1.8 and 1.9).

A closer examination of Wilson's data suggests that the relationship can be described by an equation

$$y = 10^{-(x + c)} + D$$

where  $x$  = log % organic content, and  $y$  = pH

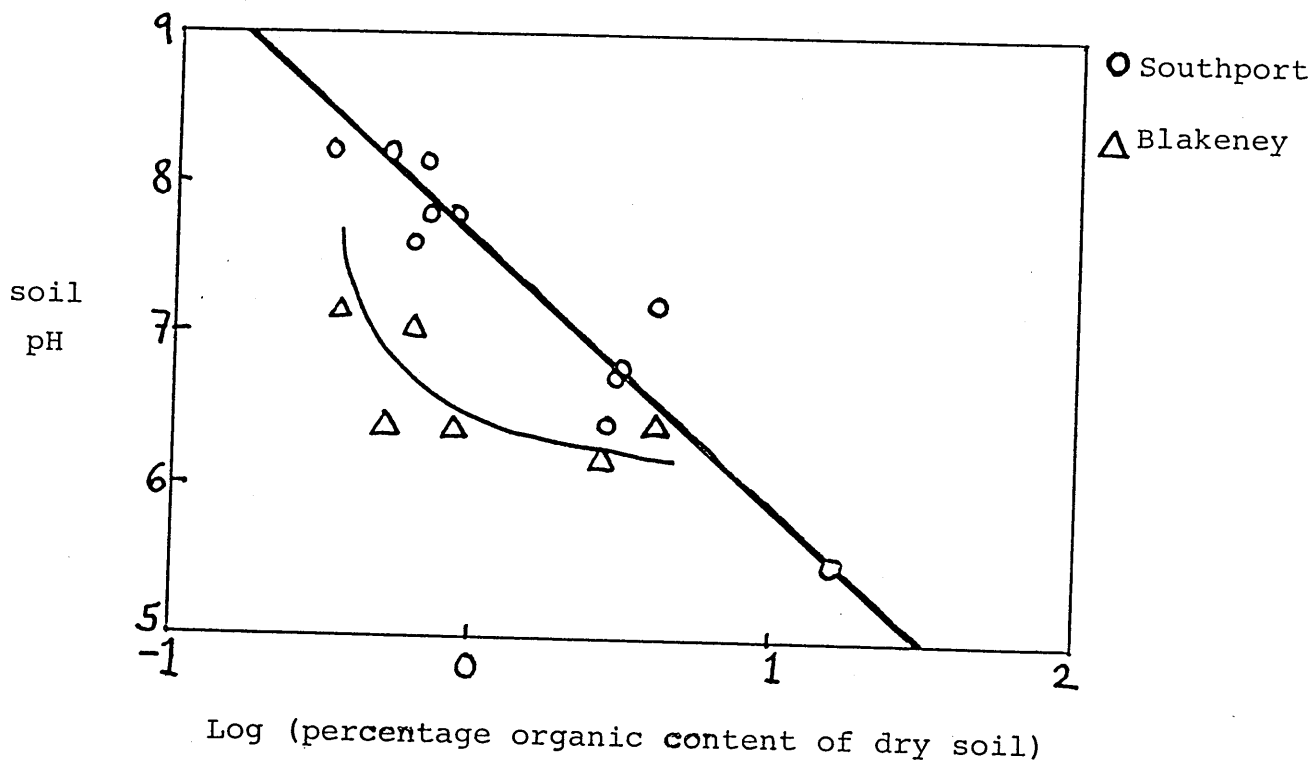
$C$  and  $D$  are constants

Table 1.3 Effect of organic matter on dune soil pH

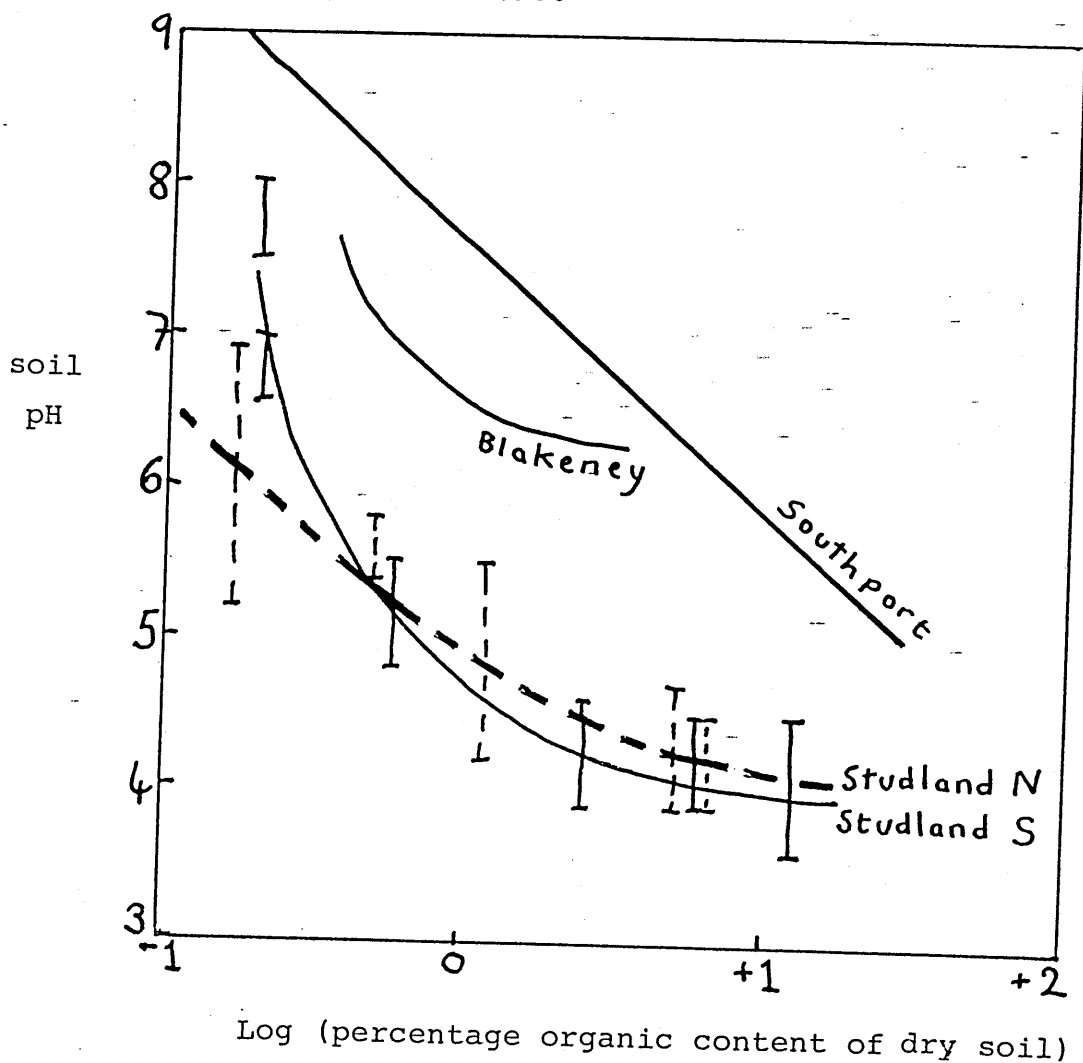
Southport (SALISBURY 1925)				Blakeney (SALISBURY 1922)				Studland-North (WILSON 1960)				Studland-South (WILSON 1960)			
pH	% O	Log		pH	% O	Log		pH	% O	Log		pH	% O	Log	
8.2	.528	0.28		7.17	0.36	0.44		8.00-	0.20	0.70		6.9-5.2	0.17	0.77	
8.2	.335	0.48		7.03	0.50	0.30		7.5				5.4-5.8	0.50	0.30	
8.15	.68	0.17		6.4	0.53	0.28		7.0-6.6	0.21	0.68		5.5-4.2	1.14	0.06	
7.8	.70	0.16		6.4	0.86	0.07		5.5-4.8	0.57	0.24		4.7-3.9	5.12	0.71	
7.6	.63	0.20		6.38	1.15	0.06		4.6-3.9	2.54	0.41		4.5-3.9	6.63	0.82	
7.8	.88	0.06		6.24	2.69	0.43		4.5-3.9	5.98	0.78					
7.2	4.00	0.60						4.5-3.6	12.67	1.10					
6.4	2.70	0.43													
6.8	2.92	0.47													
6.8	2.86	0.46													
5.5	15.0	1.18													

% O = average percentage organic content by weight      log = Log % O

Graph 1.8 Comparison of organic content and pH value of dune soils from different sites (data from Salisbury 1922, 1925) 27



Graph 1.9 Comparison of organic content and pH value of dune soils from different sites (data from Salisbury 1922, 1925, Wilson 1960)



Taking the regression line for the North Studland data from Graph 1.9 it was possible to derive values for the two constants and hence indicate the agreement between observed and predicted values from the equation. This is shown in Table 1.4 below and this regression equation is shown in Graph 1.10 against the field data .

Table 1.4 Relation between organic matter and pH of dune soil (data from Wilson 1960)

Regression line on graph	observed observed predicted	x y <sub>1</sub> y <sub>2</sub>	0.75 7.3 7.5	0.5 5.9 5.5	0.25 5.1 5.1	0 4.7 4.6	0.5 4.2 4.2	1.0 4.0 4.1
Raw data	observed observed predicted	x y <sub>1</sub> y <sub>2</sub>	0.70 8-7.5 7.2	0.68 7-6.6 -	0.24 5.5-4.8 5.1	0.41 4.6-3.9 4.25	0.78 4.5-3.9 4.10	1.10 4.5-3.6 4.05

KEY x = Log (% organic content)

y<sub>1</sub> = observed pH values

y<sub>2</sub> = predicted pH =  $10^{-(x + 0.2)} + 4$

Hence values for C = 0.2 and D = 4

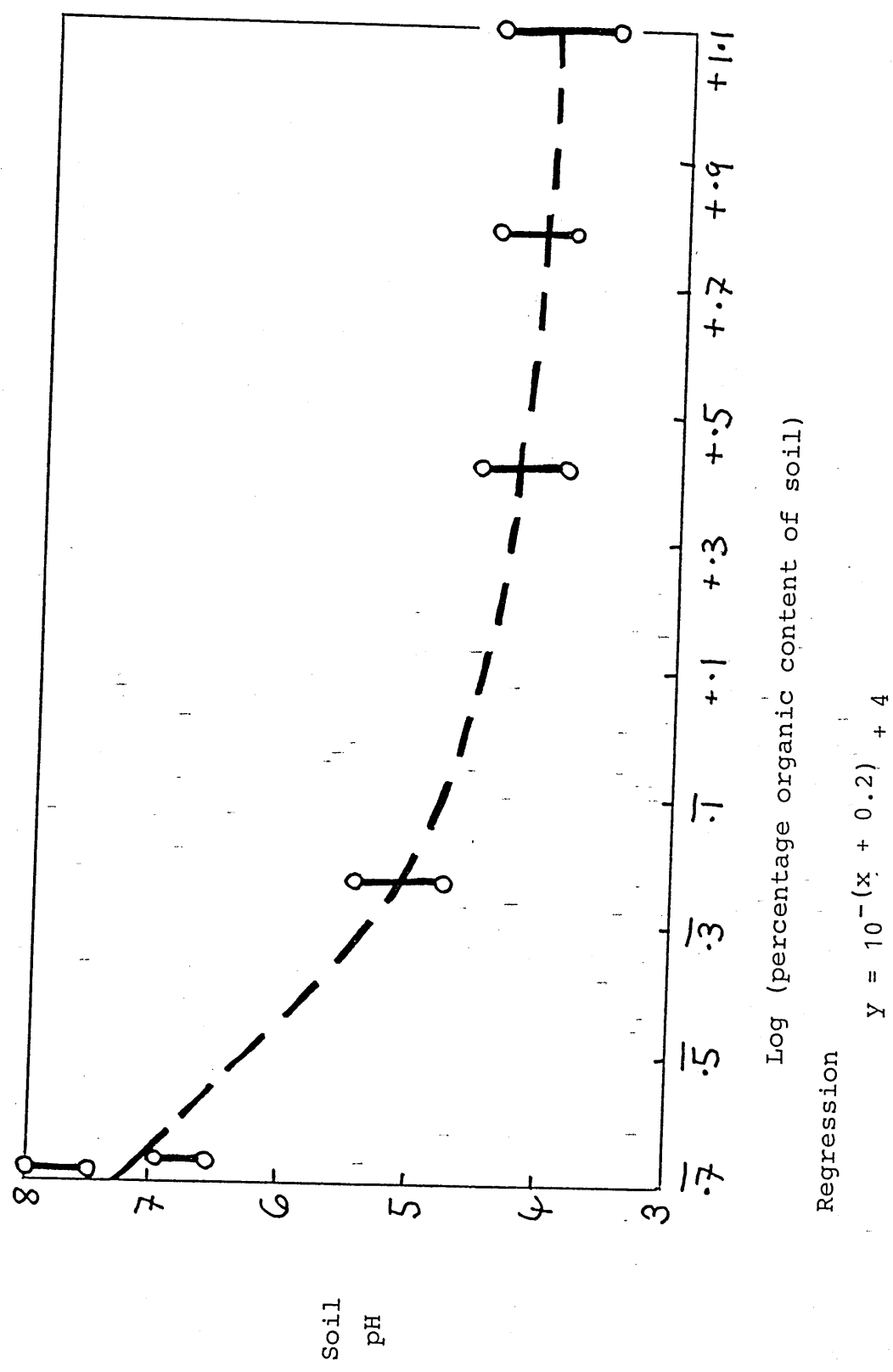
The essential point about these analyses is that as organic content increases in dune soils it gradually reduces pH to a minimum value, independent of further increases. This minimum level corresponds to the constant D in the regression equation.

Its value is about 4.0 at Studland, a distinctly "acid" dune system, with acid-loving plants like heather, and sundew. At Blakeney, a more "neutral" system the value of D is about 6.0.

The precise reasons for these different equilibrium, or minimum values for pH are not clear. Salisbury (1925)



Graph 1.10 Formal relationship between organic content and pH value of dune soils (data from Wilson 1960)



suggested that rainfall might be a factor in the contrast between Southport and Blakeney. The rainfall is similar at Southport and Studland (30"-40" per annum) while Blakeney has much less (< 25" per annum).

The significance of the results for this study are that pH in a non-carbonate dune soil is conditioned by the release of organic acids from surface litter. It would seem likely that the release of soluble nitrates probably follows a similar pattern.

When nitrogen-fixers like the sea buckthorn scrub are present, this simple pattern is probably disturbed. This perturbation should be apparent in this current study.

## 1.2 Nitrogen deficiency and plant growth in dune soils

Braunton Burrows is a large dune system in north Devon, with a variety of dune habitats between the shore and the surrounding pasture land. A detailed study of the topography, climate, and soils was made during the late 1950s (Willis et al 1959).

In making soil analyses the authors noted the contrast between the mobile dunes which lack any humus layer or distinct soil profile, and the dunes in sheltered areas which show definite soil development. Two distinct types were recognized. On the dry dunes there was little accumulation of humus, and the profile showed a narrow (5-15cm) band of humus-stained sand. In the dune scrub a more definite profile was evident, with a surface layer (3-4 cm) of humus, and a dark accumulation zone (5 cm) at a depth of about 30 cm. Within the dune slacks and hollows where the water table is high with respect to the soil surface, the authors found a tendency to gleying and compaction.

The soil chemical analyses were made at a range of sites, both dry dunes, and the wetter slacks and hollows. In general these dunes are distinctly alkaline with a pH range of 8.0 to 9.0, and a carbonate content of 8.5% to 20%. The usual phenomenon of association between increase in organic content, and fall in pH was observed, together with the decrease in carbonate content. The authors confirm the observations of Salisbury (1952) that at Braunton, leaching does not produce an acid soil. The most interesting aspect of the soil analysis is the data for a wide range of ions, including calcium, magnesium, sodium, potassium, carbonate, phosphate, and chloride. Organic carbon and total nitrogen were also estimated.

Table 1.5 below is a simplified version of the data published by Willis and his co-workers, the habitats being arranged in order of increasing stability.

Table 1.5 Chemical analysis of dune soils at Braunton Burrows, Devon 1956 (from Willis et al 1959)

Dry Dunes							
	Young Dunes			Medium Dunes			Dune Scrub
pH	9.05	9.06	8.79	8.70	8.66	8.60	8.18
Organic C mg	0.52	0.19	0.95	0.74	2.44	4.47	12.60
Total N mg	0.18	0.11	0.20	0.23	0.41	0.67	2.15
Carbonate mg	119.6	115.4	103.9	92.8	98.2	78.4	51.0

Slacks and Hollows							
	young	medium				scrub	
pH	8.99	8.73	8.42	8.22	8.12	8.11	8.06
Organic C mg	0.41	1.39	5.82	9.23	22.93	13.55	19.47
Total N mg	0.15	0.26	0.81	1.36	2.38	1.38	2.82
Carbonate mg	109.7	112.1	75.9	96.6	73.5	78.3	68.4

#### KEY

Organic C mg = mass of organic carbon in mg per gram of dry weight of soil

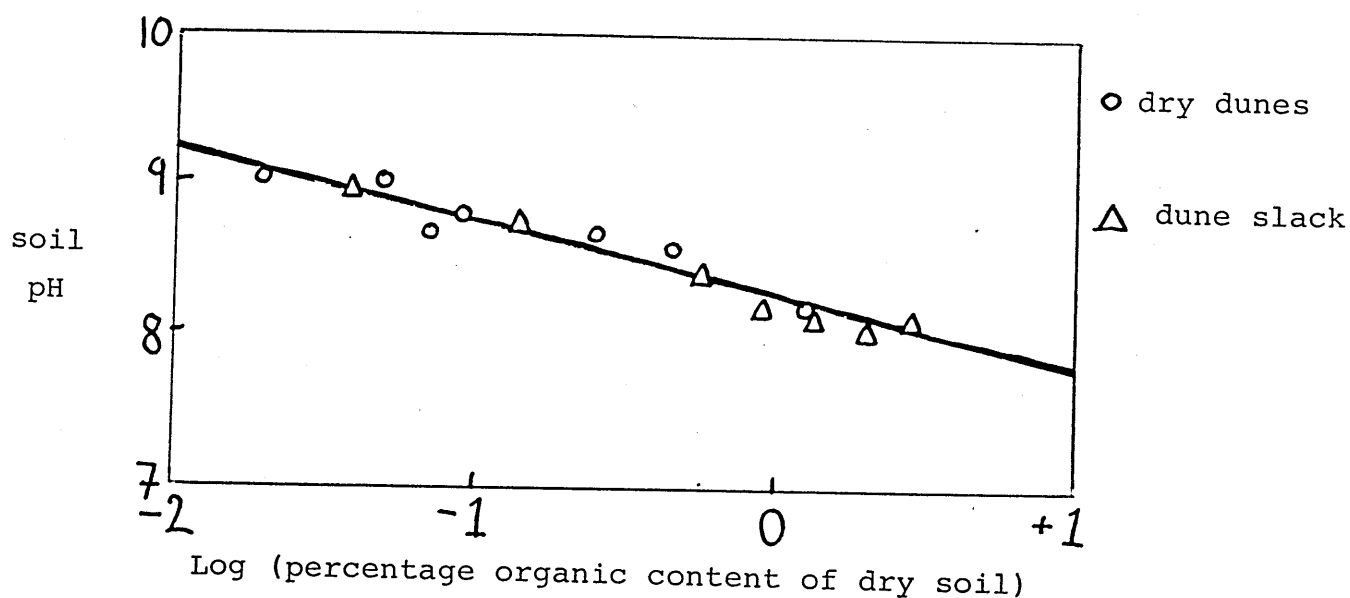
Total N mg } also expressed as mg per gram of dry weight  
Carbonate mg } soil

Table 1.6 The relation between dune-soil pH and organic matter Braunton, Devon (Data after WILLIS ET AL 1959)

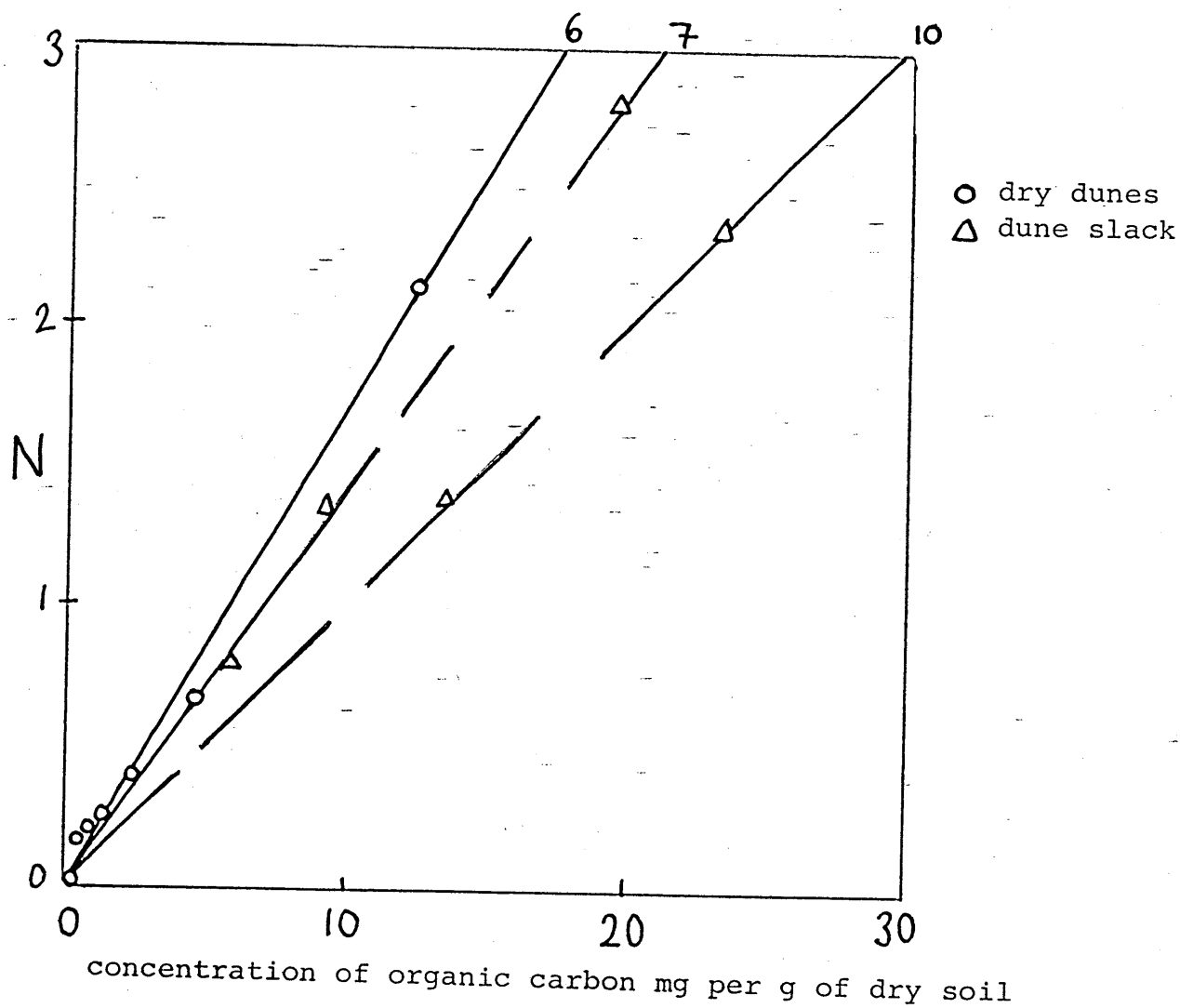
pH	9.05	9.06	8.79	8.70	8.66	8.60	8.18
Organic C mg/g	0.52	0.19	0.94	0.74	2.44	4.47	12.60
Organic C %	0.05	0.02	0.09	0.07	0.24	0.45	1.26
Log % org	$\bar{1}.30$	$\bar{1}.70$	$\bar{1}.05$	$\bar{1}.15$	$\bar{0}.62$	$\bar{0}.35$	0.10
pH	8.99	8.73	8.42	8.22	8.12	8.11	8.06
Organic C mg/g	0.41	1.39	5.82	9.23	22.93	13.55	19.47
Organic C %	0.04	0.14	0.58	0.92	2.29	1.36	1.94
Log % organic	$\bar{1}.40$	$\bar{0}.85$	$\bar{0}.24$	$\bar{0}.04$	0.36	0.13	0.29

The earlier discussion of data from Southport (SALISBURY 1925) indicated that when soil carbonate levels are high in dune systems a linear relationship exists between pH and Log (% organic content). Converting Willis's data to percentage values (Table 1.6) it is clear that a similar linear association exists at Braunton where carbonate levels also high (around 10%) at most of the sample sites. However the slope of the regression line is about a quarter of that for Southport. (Graph 1.11) The pH decreases from about 9 to 8 consistent with alkaline nature of whole dune system.

Graph 1.11 Comparison of organic content and pH value of dune soil (data from Willis 1959) et al



Graph 1.12 Relation between the amount of organic carbon, and the total nitrogen content of dune soil (data from Willis 1959)



Key: N = concentration of total nitrogen in mg per g of dry soil

It seems likely from these analyses that pH is an expression of several distinct factors only one of which is the organic content of the soil.

In estimating total nitrogen Willis and his co-workers made use of the standard Kjeldahl digestion method. This is a particularly vigorous analytical technique and it is no indication of the nitrogen which is actually available to plants. Total nitrogen is more an expression of the continuing potential of the soil in terms of releasing soluble nitrogen compounds over a period of time. The plot of total nitrogen against total organic carbon (Graph 1.12) shows a broadly linear relationship. There seems to be a distinct difference between the dry dunes and the wetter slacks in that the ratio  $\frac{N_t}{O_t}$  is greater in the former.  $N_t$  = total nitrogen  
 $O_t$  = total organic. It is not clear why this should be so. Certainly the pH change is too small to account for the difference.

Although the soil analysis data do not include nitrates or other soluble nitrogen compounds Willis and his team were interested in both nitrates and phosphates and the effect of their absence on soil fertility, and the dune succession. They considered that "the soils initially low in certain nutrients may be further depleted by leaching, and it therefore seems probable that the growth of plants on dunes is often limited by soil fertility" (WILLIS & YEMM 1961 p. 377).

Their experiments were largely concerned with the effects of augmenting the minerals already present in dune soils, on the growth of selected plants within a greenhouse environment. It

was quickly evident that only culture solutions which added nitrogen or phosphorus promoted successful growth in dune soils. The rate of growth of control plants utilizing the very low natural levels of N or P were apparently much the same as those given N-deficient or P-deficient culture solutions, although no data for this particular contrast were published. (p. 385)

The results led to the conclusion that both nitrogen and phosphorus together were needed for effective plant growth. A further series of experiments showed that phosphorus was the principal growth-rate limiter. In these tests, a series of natural dune-turf transplants were grown under greenhouse conditions and watered with particular culture media. Willis and Yemm were mainly interested in the changes in relative abundance of the various species present in the turf transplants. I have re-presented their data in an abbreviated form which concentrates on the success of plant growth under different nutritional regimes. (Table 1.7)

In making use of the data in Table 1.7 a certain amount of caution is needed. The species of plants present at the three different sites varied considerably (indeed they were chosen for this reason) so that it is not valid to try to make comparisons between different sites solely in terms of general plant growth. It is the variation in species response (discussed at length by Willis and Yemm) which is significant.



Table 1.7 Plant growth on dune soils following treatment with nutrient solutions - Braunton Burrows, Devon. (Data from WILLIS & YEMM 1961)

Treatment		Control			All Nutrients			N + P + K			NO <sub>3</sub> <sup>-</sup> o		
Time - weeks		0	15	38	0	15	38	0	15	38	0	15	38
DP	Av Ht Wt	2	2.5	3.5 24	2	13	13 77	2	13	13 67	2	6	8 47
DDP	Av Ht Wt	3	4	6 23	3	15	17 65	3	14	16 50	3	11	12 41
LP	Av Ht Wt	2	3	7 47	2	11	15 81	2	8	17 57	2	8	12 48

KEY: Time - from the start of the experiment - weeks

Av Ht - average height of plants in cm

Wt - fresh weight of shoots in g

DP - damp pasture

DDP - dry dune pasture

LP - lichen pasture

(The fresh weight is a harvest technique so data are only available for the end of the experiment)

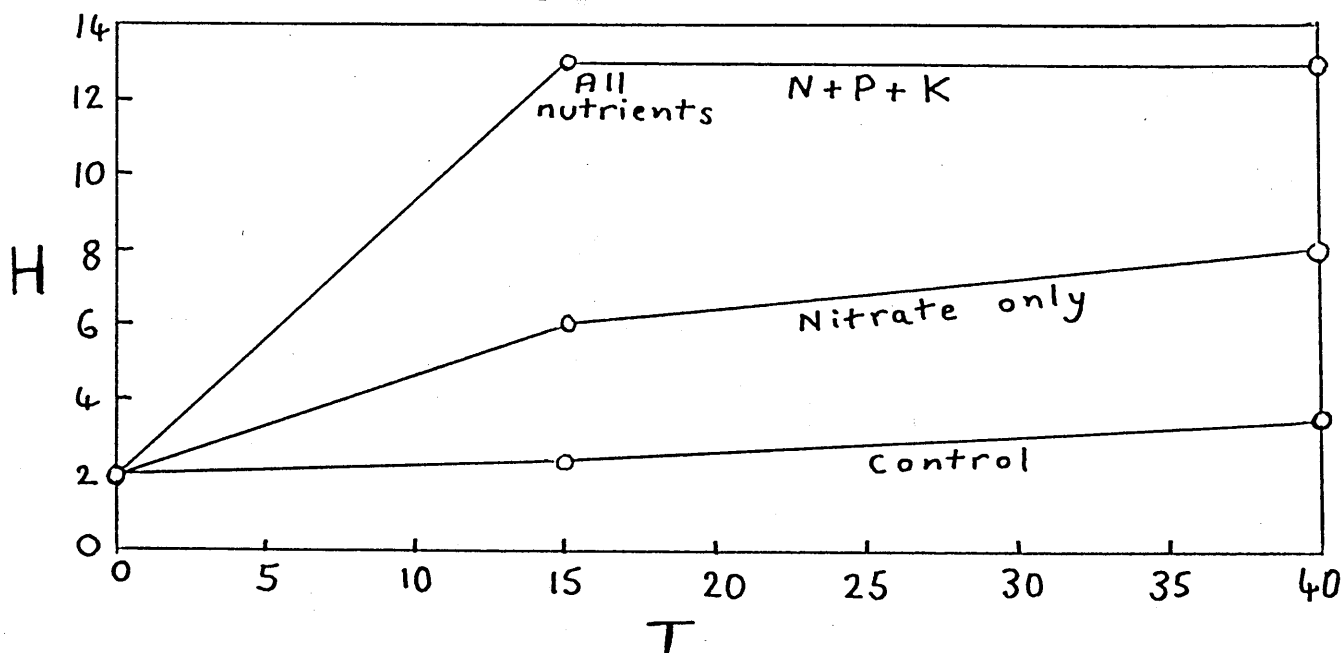
Comparisons between treatments at a given pasture type are valid. Willis and Yemm did not use replicates for each pasture type X treatment. Given the fact that they were using a wide area each set of figures, (on height for example), represents a mean of a considerable number of individual plant responses, to growth nutrients.

Plant growth on dune soils following treatment with nutrient solutions -  
 Taunton Burrows, Devon. (Data from Willis and Yemm 1961)

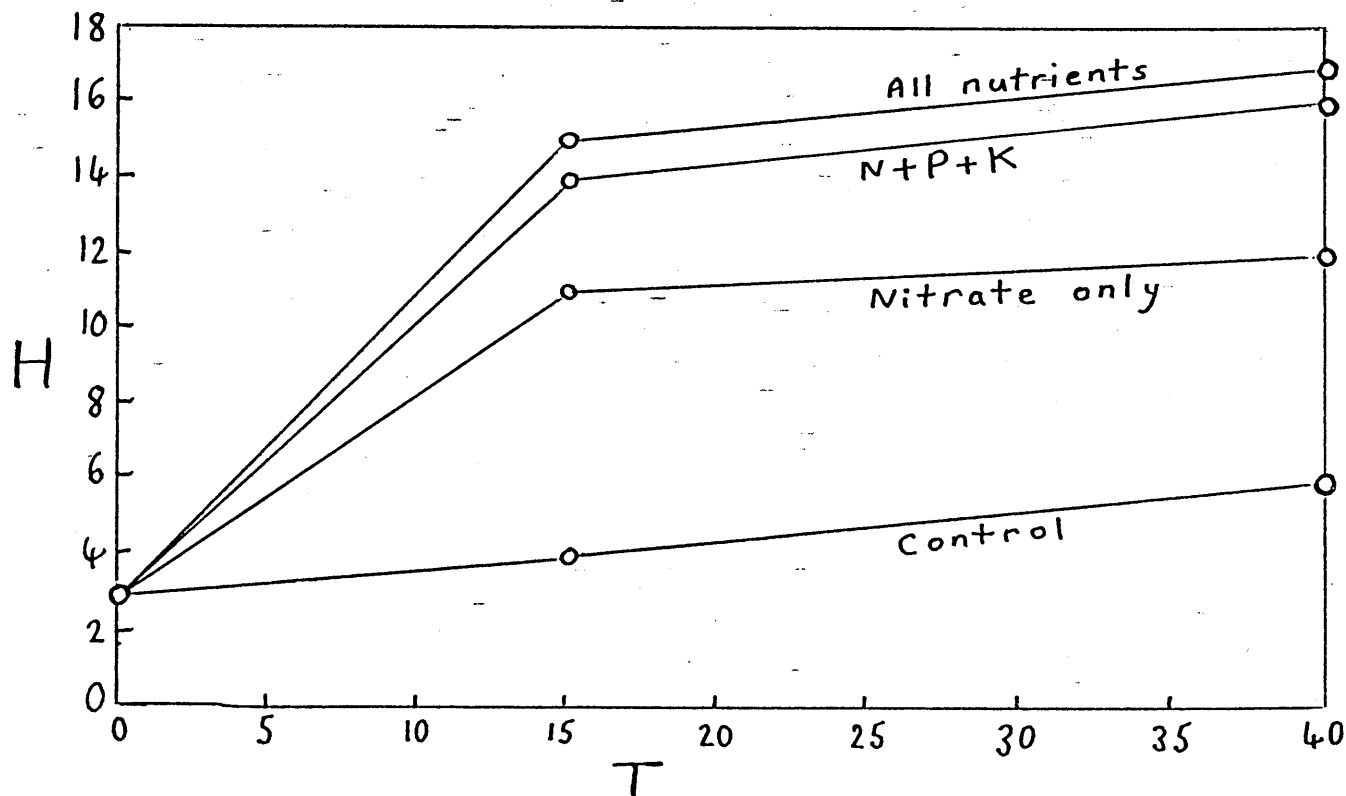
Graphs 1.13 to 1.15 Key H = average plant height in cm

T = time from start of experiment in weeks

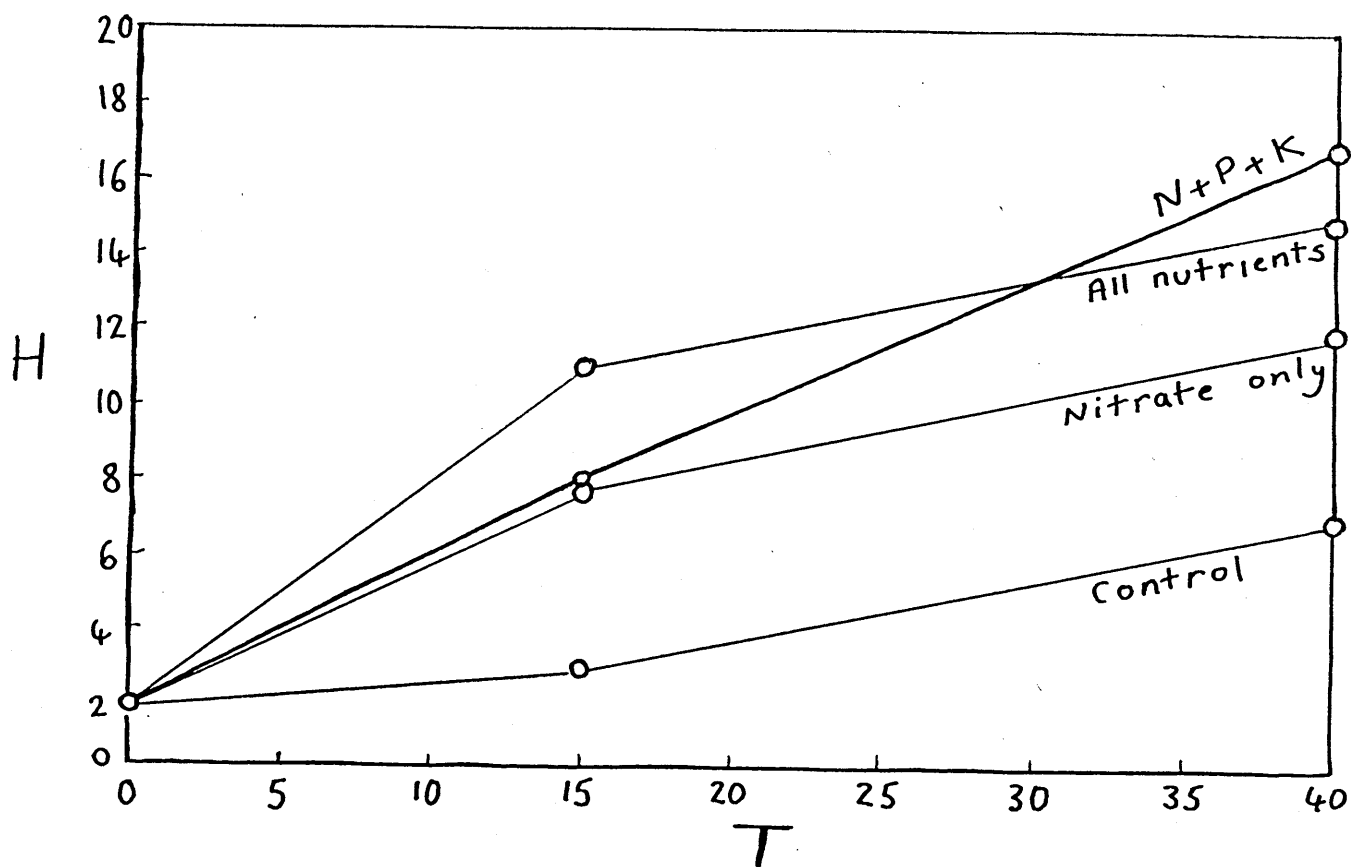
Graph 1.13 Damp pasture



Graph 1.14 Dry dune pasture



Graph 1.15 Lichen pasture



These responses are shown in Graphs 1.13, 1.14, and 1.15, based on data in Table 1.7. It is immediately evident that the controls showed signs of growth in all three pasture types. Although the dune soil may lack nutrients it is clear that the plants which establish themselves there naturally can cope with adverse conditions, and can make limited growth.

The growth achievements of the plants supplied with all nutrients, were similar to those plants supplied only with N, P and K. This indicates that there are sufficient of the minor nutrients for growth. It is the major nutrients which are rate-limiting in these soils, on all of the pasture types.

Finally, it is clear that supplying nitrate alone does not bring about as much improvement in growth as when the nitrogen is present with phosphorus or potassium. The evidence suggests that phosphorus itself is the key nutrient here. Willis and Yemm reported the physiological signs of phosphorus deficiency in many of the plants used in the study. It is interesting that, although the soil is rich in carbonates, derived from sea-shells, which also provide calcium and magnesium phosphates, the plants derive no benefit from this. The authors suggest that "poor exploitation by the roots may in part account for the acute phosphorus deficiency observed, despite the fact that chemical analysis indicates that phosphorus is present in the soils in considerably greater quantities than potassium. It is probable also that not all of the total phosphorus is in a form readily available to plants" (WILLIS & YEMM 1961 p. 389).

My own measurements of soluble phosphate levels at Holme showed them to be very low (between zero and 0.09 ppm dry

soil,) despite the presence of insoluble phosphate in shells at around 2% total carbonate.

The observations of Willis and Yemm on phosphate form a parallel to my earlier comments on "total nitrogen". Their measurements of phosphate are little more than a statement of the unrealised potential of the dune soil. They found plants dying of phosphorus deficiency while surrounded by insoluble soil phosphates. It is clear that the measurement of soluble phosphate and available nitrogen are the key issues in examining the nutritent status of dune soils.

As a check on the general validity of the results, the relationship between the fresh weight, and the average height at 38 weeks was examined. Broadly, one would expect the three experimental treatments to yield similar proportions in the results for each of the two parameters, for a given pasture type. For ease of comparability, both sets of results were reduced to a common standard based on the control. So  $W_s$  = fresh weight of shoots, for experimental treatments, as a proportion of the control fresh weight as unity, and  $H_s$  = average height of plants at 38 weeks, for experimental treatments as a proportion of control average height at 38 weeks as unity.

Table 1.8 shows these results and Graphs 1.16, 1.17, and 1.18 illustrate the plots of  $W_s \times H_s$  for the three pasture types.

Table 1.8 Plant growth on treated dune soils: comparison of standardized fresh weights and average heights -  $W_s$  and  $H_s$  (data from WILLIS & YEMM 1961)

PARAMETER	PASTURE TYPES	NUTRIENT TREATMENTS			
		CONTROL	ALL	NPK	NO <sub>3</sub> ONLY
$W_s$	DP	1.0	3.2	2.8	2.0
	DDP	1.0	2.8	2.2	1.8
	LP	1.0	1.7	1.2	1.0
$H_s$	DP	1.0	3.7	3.7	2.3
	DDP	1.0	2.8	2.7	2.0
	LP	1.0	2.1	2.4	1.7

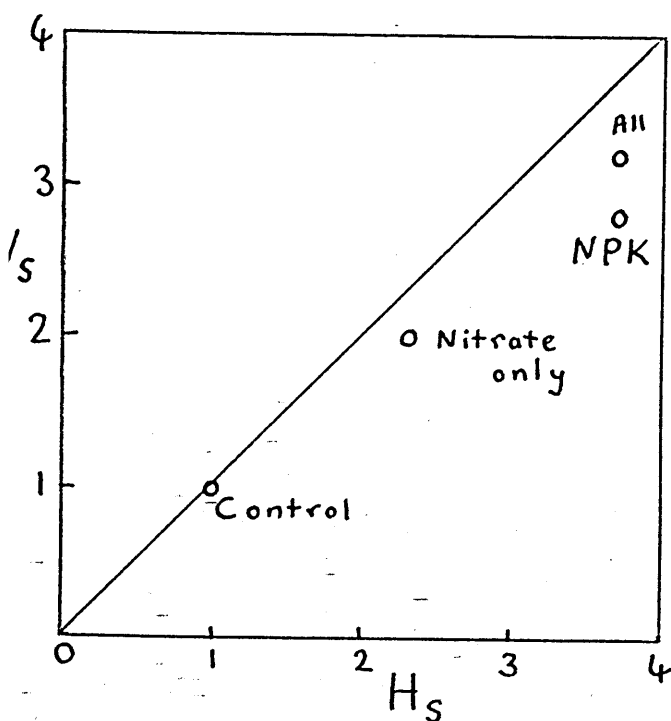
The graphs demonstrate that the growth proportions tend to be lower with respect to  $H_s$ , or more simply the measurement of height probably under-estimates the actual amount of growth made, compared with measurements of weight. The discrepancies are not great, and the results of harvest of fresh weights confirms the picture obtained by height measurements.

In connection with the study of dune soil nitrogen, the two most important results emerging from the studies by Willis and Yemm, are (i) that despite low levels of soluble phosphate and nitrate natural colonizers do manage to grow, and (ii) that estimates of nutrients that are readily accessible to plants are a crucial aspect of any examination of soil fertility.

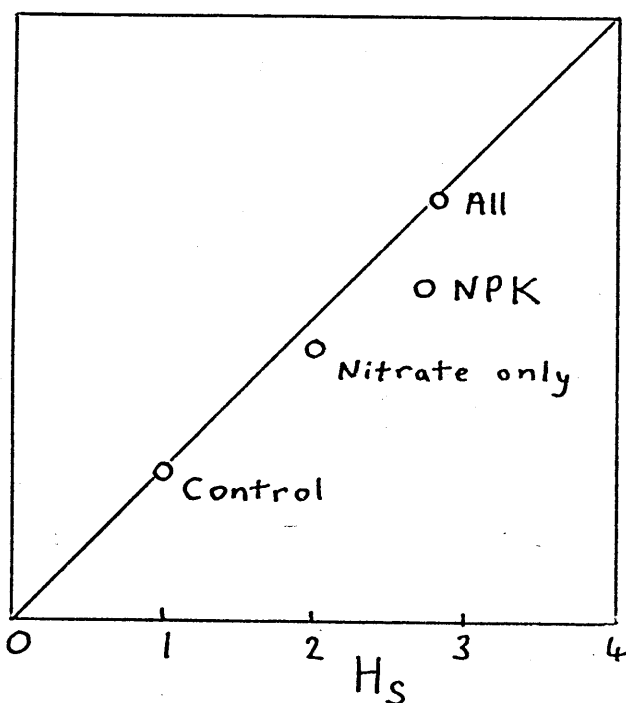
Comparison of standardized fresh weights in g ( $W_s$ ) and average heights, in cm ( $H_s$ ) of plants grown on three types of dune soil, with four types of nutrient solutions - Branton Burrows, Devon.

(Data from Willis and Yemm 1961). Graphs 1.16 to 1.18

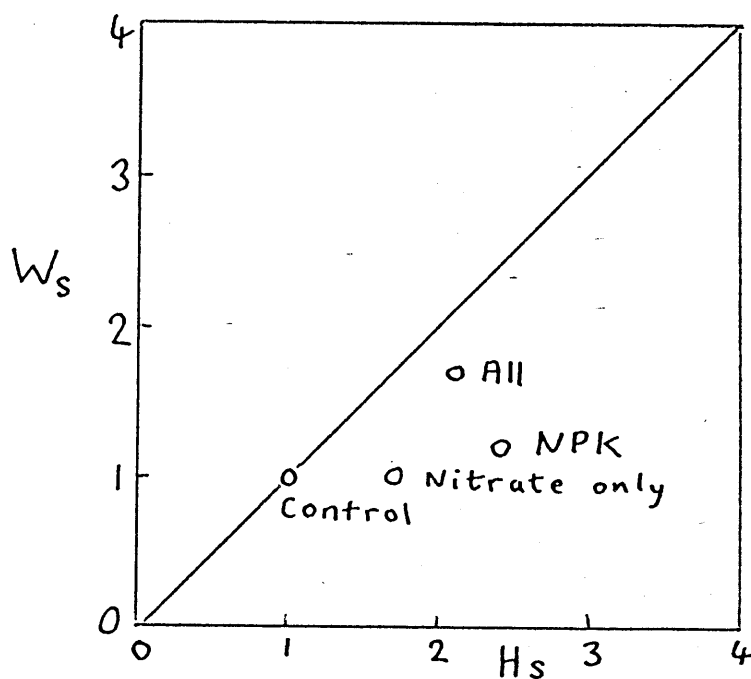
Graph 1.16 Damp pasture



Graph 1.17 Dry dune pasture



Graph 1.18 Lichen pasture

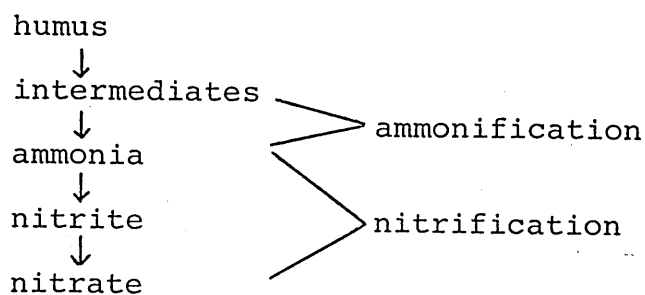


- 1.3 The liberation of soluble nitrogen compounds from organic matter in dune soils
  - 1.3.1 Origins of the incubation technique
  - 1.3.2 Biological activity
  - 1.3.3 Experimental methods
  - 1.3.4 Formation of ammonia
  - 1.3.5 Formation of nitrate
  - 1.3.6 Daily nitrate production
  - 1.3.7 Differential bacterial distribution
  - 1.3.8 Nitrogen fixation
  - 1.3.9 Low nitrate production
  - 1.3.10 Mineralization ratios
  - 1.3.11 Total nitrogen



### 1.3 The liberation of soluble nitrogen compounds from organic matter in dune soils.

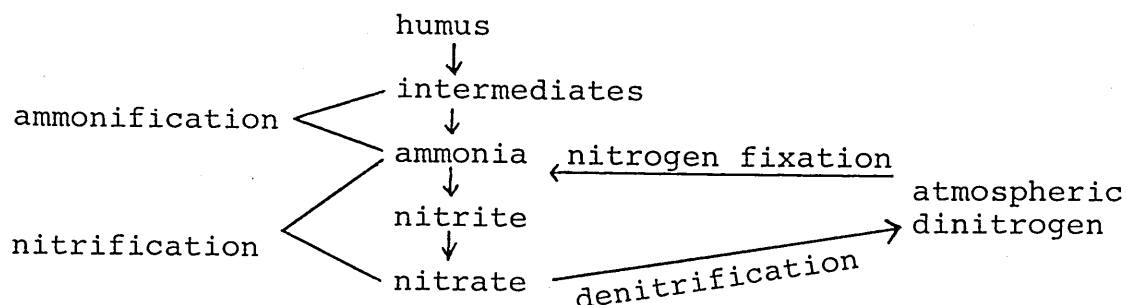
As it has been shown that nitrogen-deficiency can occur in dune soils containing appreciable levels of organic matter, it is important to examine how that organic matter breaks up to liberate simple soluble nitrogen compounds available to plants. This process is termed "the mineralisation of organic nitrogen" and was initially seen to consist of two linked processes ammonification and nitrification.



The process of mineralisation has been studied by collecting soil samples, incorporating their usual bacterial and other microflora, and incubating them over several weeks while regularly monitoring the production of soluble nitrogen compounds. This was the experimental basis of an important paper on the nitrogen-economy of coastal soils in the Pas de Calais area of northern France (GÉHU and GHESTEM 1965).

The paper deals with both salt-marsh and sand-dune soils but in the following analysis I have limited myself to <sup>the</sup> section on dunes. In particular I have drawn attention to the problem of nitrogen-fixation. This might have been a source of error in the incubation technique and needs to be taken into account when discussing results. The authors do not mention nitrogen-fixation directly in their commentary. The significance of

the process is indicated in an extension of the earlier diagram.



### 1.3.1 Origins of the incubation technique

The authors point out that although the incubating technique was proposed as long ago as 1926 by Hesselmann and was widely used by agronomists in the study of cultivated soils, it was many years before it was employed in the study of "natural" or non-cultivated soils. During the period 1953-1963 a series of studies of the nitrogen-economy of forest humus soils was made, but coastal soils were largely neglected. Géhu and Ghestem were able to extend the scope of the incubation technique when they applied it to salt-marsh and sand-dune soils. They criticised the failure of earlier workers to relate incubation studies to a proper floristic analysis of the habitats studies. Consequently there is a brief but valuable phytoecological description of the soil sample sites used in the study.

This proves to be of great significance when examining the results obtained by Géhu and Ghestem especially when considering the possibility that nitrogen-fixation may have played a part.

### 1.3.2 Biological activity

There is a reference to "biological activity" in Hippophaë which may mean nitrogen-fixation but the phrase is too vague for this interpretation to have any certainty. The original paper states "Pour ces différents milieux, on remarquera encore la réduction assimilative des nitrates, plus ou moins prononcée, et décelable surtout la première quinzaine. Dans l'Hippophaetum le phénomène se trouve masqué en raison de l'activité biologique considérable." (GÉHU & GHESTEM 1965 p. 141).

I have translated this as "In these different situations, it is noticeable once again how the assimilative reduction of nitrates, is detectable within the first fifteen days. In the Hippophaetum this phenomenon is masked because of considerable biological activity."

### 1.3.3 Experimental methods

The authors recognised nine different dune communities and used these as the basis for their soil sampling distributions. An abbreviated form of their descriptions is given below. A reference letter and an English title have been added to each, to facilitate all subsequent tabular and graphical presentations of their data.

- A. Upper beach (Cakiletum arenariae)
- B. Fore dune (Elymeto-Agrophyretum)
- C. Yellow or mobile dune (Euphorbieto-Ammophiletum)
- D. Dune pasture (Festuco-Galietum)
- E. Fixed or grey dune (Tortulo-Phleætum)

- F Buckthorn scrub (Hippophaeto-Ligustretum)
- G Neutral pasture (Corynephorretum)
- H acid pasture (Festuco-Thymetum)
- I acid heath (Calluno-Genistetum)

In their introductory comments the authors discuss the various chemical processes occurring within the dunes of their study area, and it is evident that these are identical with those described for comparable dune systems around the British coasts.

The experimental methods used were simple but meticulous. Soil samples were collected from the top 10 cm of each of the nine habitats A to I and twelve replicates were taken at each habitat. The samples were sieved, homogenised, humidified and incubated at 25 °C for ninety days. Estimations of nitrate and ammonia were made at the start, and subsequently on days 15, 30, 45, 60 and 90. The data were presented in the paper as histograms rather than graphs, as the authors considered the latter would have been too confusing given that sequences of two different parameters were involved.

Fortunately the histograms are sufficiently detailed to enable tables to be prepared from them. This was of particular importance when determining the amounts of total nitrogen. The raw data and various proportions derived from them are presented in Table 1.19 and the main trends emerging from them are shown in Graphs 1.19 to 1.22.

Table 1.9 Variation in nitrogen as ammonium ion and nitrate ion in dune soils  
incubated at 25 °C for 90 days (data from GEHU and GHESTEM 1965)

Dune community		A	B	C	D	E	F	G	H	I
Ammonium ion	T = 0	3	2.5	3	3.5	3.5	6.5	2.5	4	2
	15	1	1	2	2.5	2.5	3	3	5.5	4.5
	30	3	2	2.5	3	2.5	3	3	16.5	16
	45	2	4.5	3	3	2	3	2	19	20
	60	2	3.5	4.5	4	2	4	2	21	29
	90	2.5	3	3	4.5	3.5	6	3	25	29
Nitrate ion	T = 0	4.5	8	8.5	8	5	18	4	12	5.5
	15	1.5	2	2	7.5	4.5	49	4	9	4
	30	9	4	8	10	6	58	6	15.5	4
	45	6.5	5	16.5	14	7.5	85	8	20.5	5
	60	8	6	23.5	17	8	107	10	25.5	9.5
	90	5.5		25	21	10.5	127	12	36	34
% I 90		+ 22	- 25	+ 194	163	110	605	200	200	518
% I D		+ 0.2	- 0.2	+ 2.2	1.8	1.2	6.7	2.2	2.2	5.8

Key: Ammonium ion = concentration of ammonium ion in parts per million of dried dune soil.  
Nitrate ion = concentration of nitrate ion in parts per million of dried dune soil.  
T = time in days from the start of the incubation.  
% I 90 = percentage increase in nitrate after 90 days incubation.  
% I D = percentage increase in nitrate per day.

#### 1.3.4 Formation of ammonia

The level of ammonia present is broadly constant for the whole of the ninety day period for seven of the nine soil sites.

(Graph 1.19) This result is not altogether surprising because ammonia is known to be a short-lived member of the mineralisation sequence. It tends to be converted fairly rapidly to nitrite and nitrate. The constant level at about 4 ppm represents a continuing turnover of organic material. The two discrepant results occurred in the two most landward communities H and I. Both of these were described as acidic by the authors. It is possible that as ammonia was produced from organic matter it was converted to ammonium salts by reaction with organic acids. These salts would accumulate instead of being converted to nitrates. The flattening of the graph after about 60 days suggests that the reservoir of organic acids was possibly becoming exhausted, and the accumulation of ammonium salts was slowing up. Further production of ammonia would result in its conversion to nitrite and nitrate. Effectively a new equilibrium was being established, within the context of a large residue of organic ammonium salts.

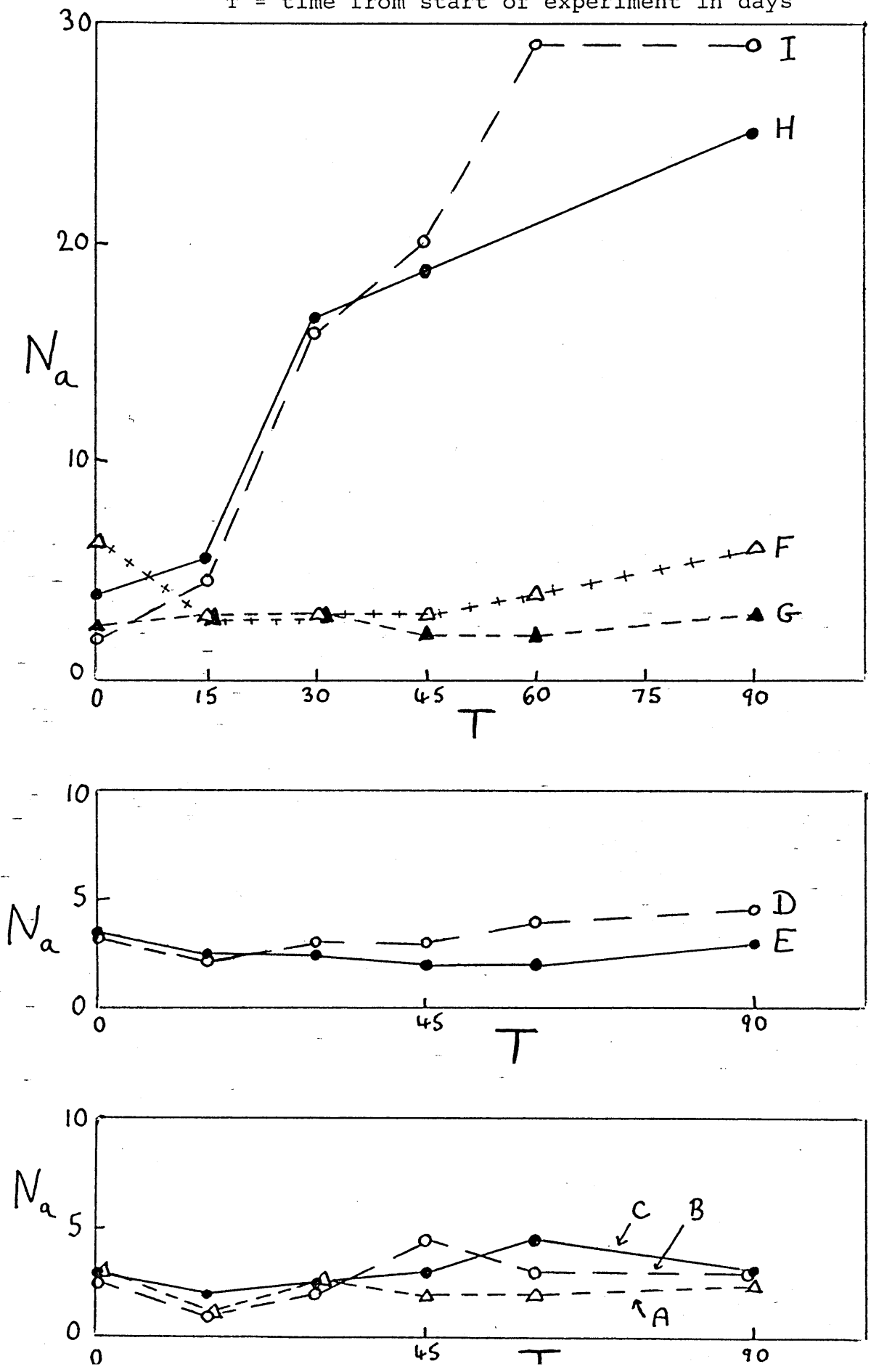
It is also possible that "some of the ammonia released during the decomposition of plant proteins...combines with quinones and polyphenols to form products of greater stability against microbial attack...it is often called ammonium fixation" (RICHARDS 1974 p. 144).

It is not possible to tell whether this explanation is correct or not without further experiment, but it may account for the fact that ammonia occurs at 6 to 7 times greater

Graph 1.19 Variation in nitrogen as ammonium ion in dune soils (A to I) incubated at 25 °C for 90 days. (Data from Géhu and Ghestem 1965).

Key: Na = nitrogen as soluble ammonium in parts per million by weight of dried soil

T = time from start of experiment in days



amounts in the acid soils. The authors limited themselves to a description of the observed effect and the comment that this was "in agreement with what we know of the development of raw humus" (p. 140).

If nitrogen-fixation was occurring a similar result might be expected. The ammonia produced could be routed into ammonium salts instead of being oxidized to nitrite and nitrate. This part of the experiment does not enable a contrast between mineralisation or fixation to be demonstrated.

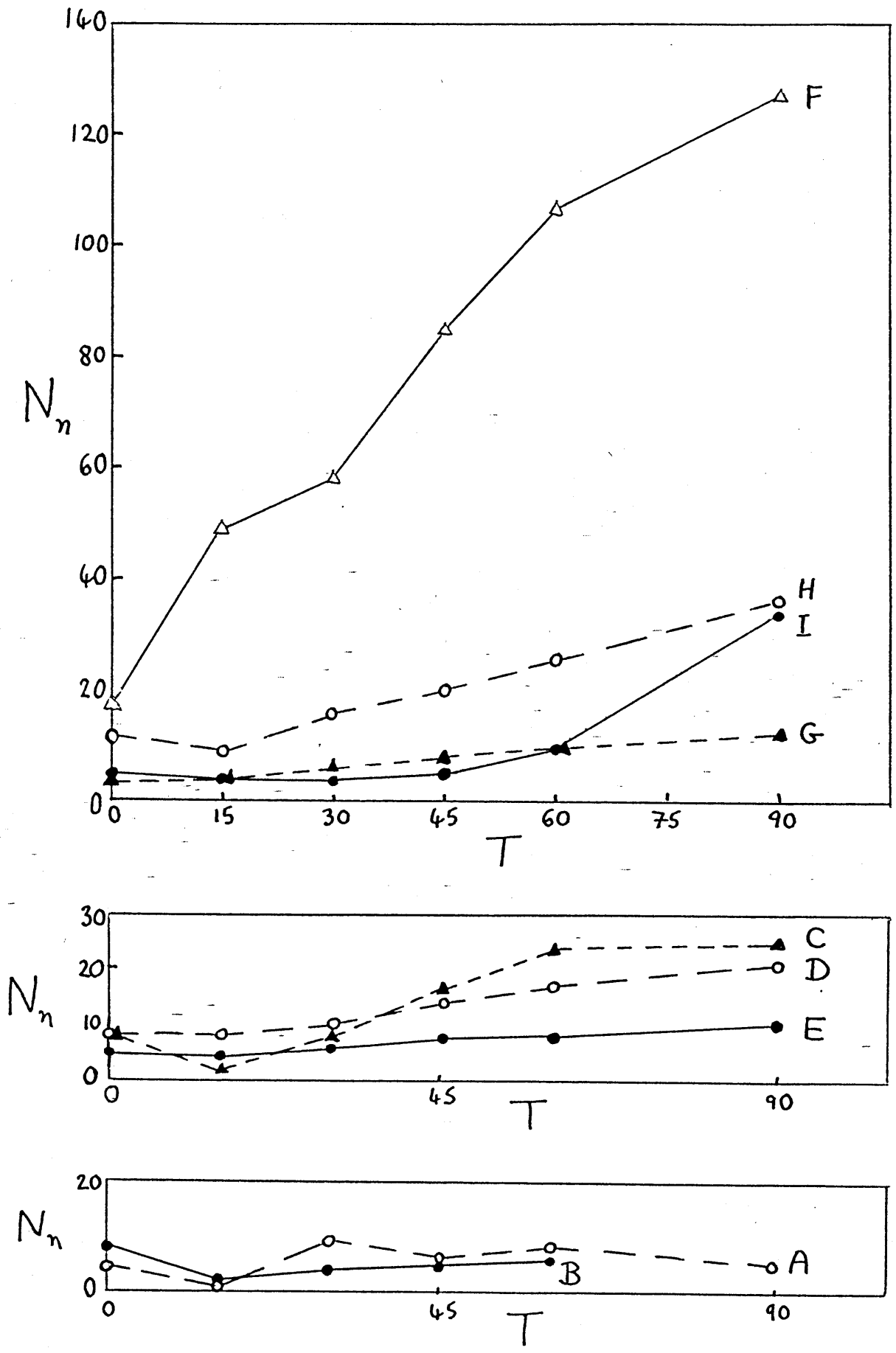
#### 1.3.5 Formation of nitrates

The results for nitrate levels over a 90 day period show a wide range of differences between one soil site and another. In almost every case there are near-linear increases in nitrate level with time. Presumably the actual production of nitrate remains constant for each soil; what the graph records is an accumulation of nitrate with time. (Graph 1.20) Examining the trends, we find that A and B show virtually no increase in nitrate over the 90 day period, and E and G make only a slight increase. Soils C, D, H and I produce a marked increase in nitrate, and F shows a spectacular increase quite different from the rest. Given that all these soils come from a single sand-dune system how can these remarkable differences be explained? Are they due to different amounts of organic matter initially present in the soil samples, or do the bacterial populations of the soil show a differential zonation as marked as that shown by the higher plants?



(A to I) incubated at 25 °C for 90 days. (Data from G  hu and Ghestem 1965).

Key:  $N_n$  = nitrogen as soluble nitrate in parts per million by weight of dried soil  
T = time from start of experiment in days



There are no data available on the amount of organic material present in each of the habitats A to I so it is not possible to make a direct comparison with the levels of nitrate recorded. However it is possible to make an indirect comparison. The sequence A to I which goes from the beach towards the land, should show a general pattern of increase in the level of soil organic matter. A plot of nitrate concentration against A-I should show linearity or at least a positive trend if there is an association between organic matter and incubation levels of nitrate. Examining Graph 1.21A there does not seem to be any clear trend.

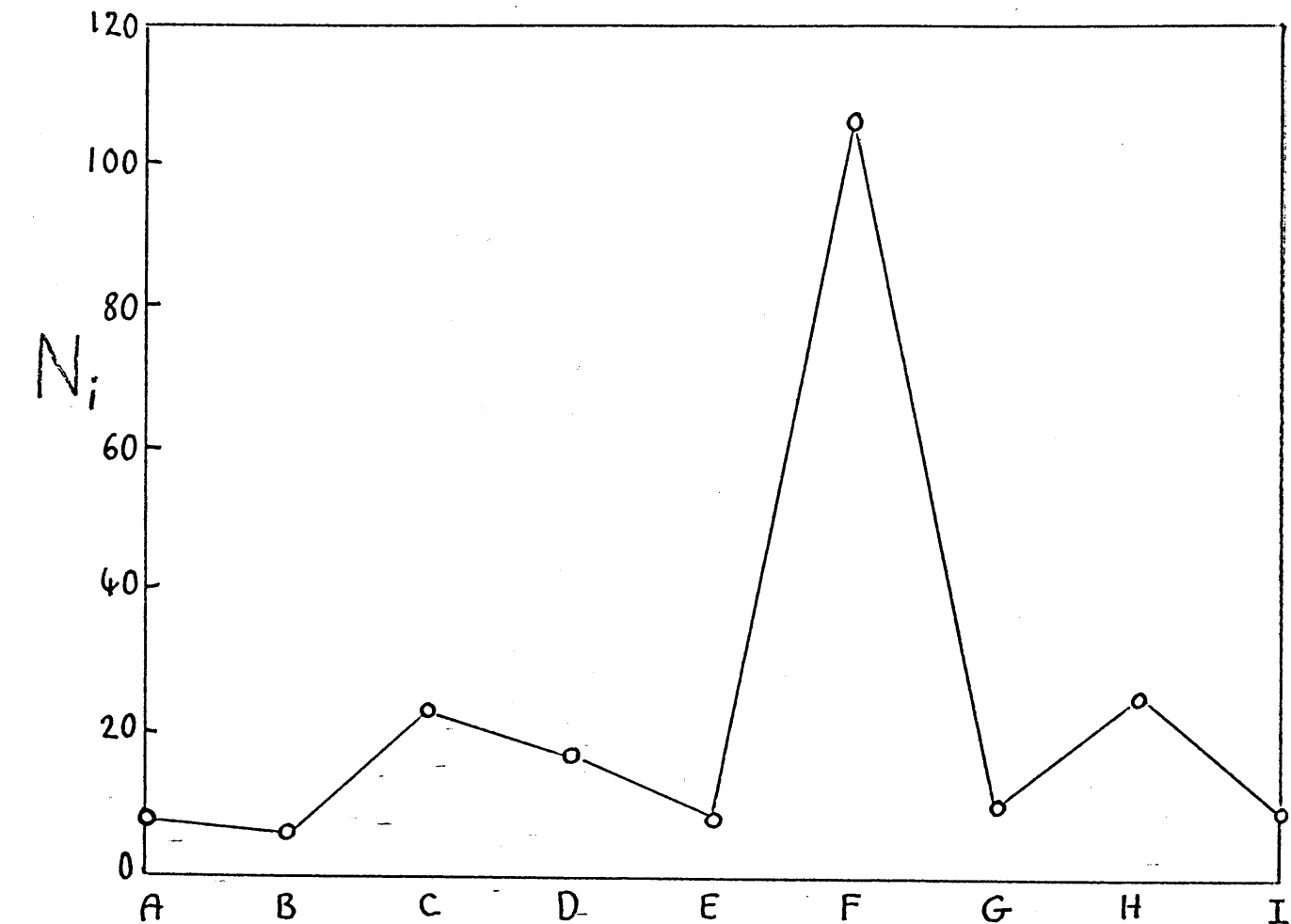
1.3.6 Daily nitrate production

It is obvious from Graph 1.20 that the soil samples all began with different levels of nitrate. By calculating the percentage increase in nitrate after 90 days it is possible to even out the starting differences between soils and concentrate on the proportional differences as a percentage of the daily nitrate production level. These data are given at the foot of Table 1.9.

When these figures are displayed in Graph 1.21B it is obvious that there are still very distinct differences between the nine types of incubated soil, but they can be resolved into three groups as follows:

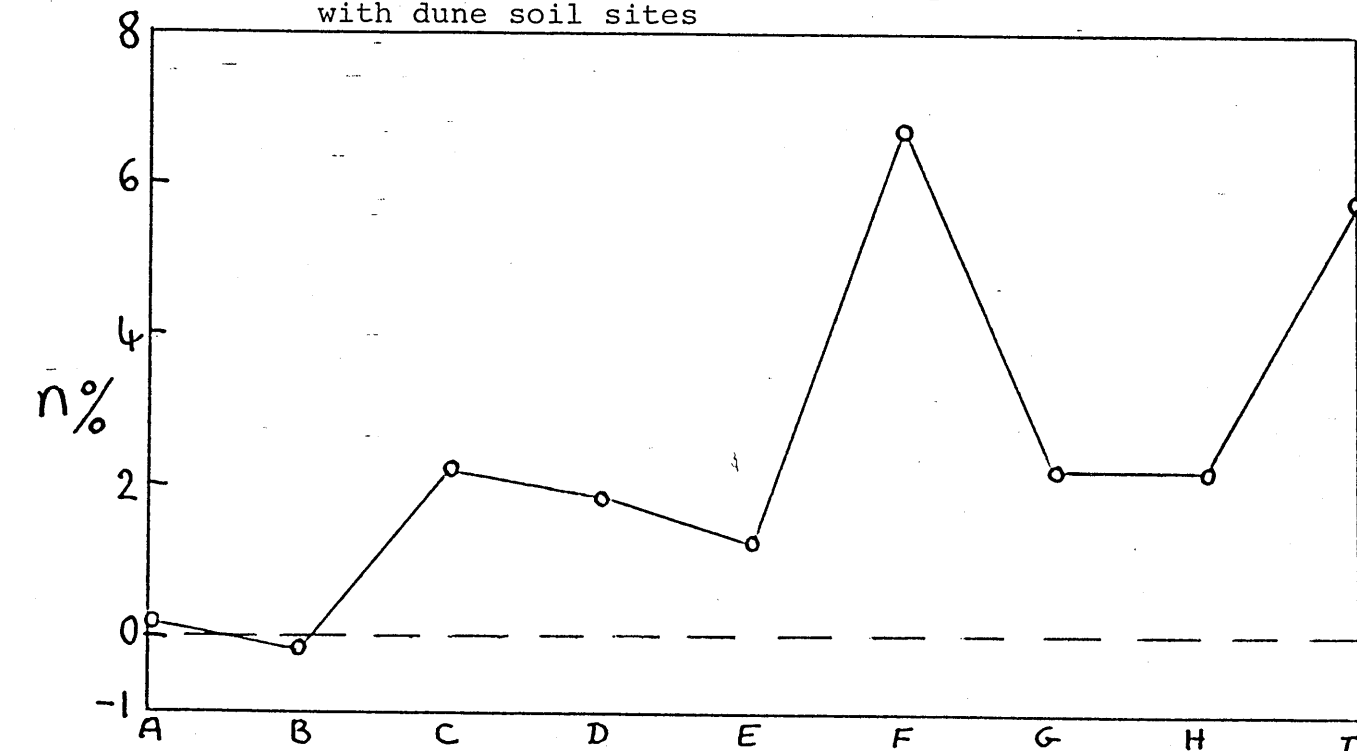
LOW	% daily increase	<0.5% $I_D$	(A,B)
MEDIUM	% daily increase	1.0 - 2.5% $I_D$	(C,D,E,G,H)
HIGH	% daily increase	>5.0% $I_D$	(F,I)

Graph 1.21 (A) Variation in nitrate after 60 days incubation, with dune soil sites.



Dune soil sites at increasing distance from the sea  
Key:  $N_i$  = nitrogen as nitrate in ppm dry soil after 60 days incubation

Graph 1.21 (B) Variation in the percentage daily increase of nitrate with dune soil sites



Dune soil sites at increasing distance from the sea  
Key:  $n\%$  = percentage daily increase in level of nitrate

### 1.3.7 Differential bacterial distribution

It is not possible, without further experiments, to tell if these differences in nitrate production are due to differences in the bacterial populations present in the soil. Possible the bacteria may show a pattern of zonation in a sand dune system comparable with that of higher plants. A very thorough study of the fungal microflora of acid dunes (Studland) and alkaline dunes (Sandwich) demonstrated that "a succession of species, comparable with that of higher plants, was found to occur across the dune systems from the pioneer communities of the foreshore to the climax communities of the fixed dunes" (BROWN 1958 p. 661). It was also evident from these studies that the acid and alkaline dunes had mycofloras which were distinct from one another. Possibly the most interesting observation from the point of view of this analysis of Géhu and Ghestem's work, was the comment by Brown that the "relatively rich and active fungal population... contained few species which appeared to be confined to the dune habitat" (BROWN 1958 p. 661).

It is possible that the soil samples collected by Géhu and Ghestem also contained few bacterial species which were confined to the dune habitat. Given a difference in the distribution of the species within the whole dune community this could account for the differences in nitrate production.

### 1.3.8 Nitrogen fixation

If some of the incubated soil samples contained nitrogen-fixing organisms as well as nitrifying bacteria the final level of nitrate production would be higher than in those

samples where nitrogen-fixing organisms were absent. Might the soils with high levels of production of nitrate contain a much higher microflora of nitrogen-fixers? The two habitat sites whose soils gave highest levels of nitrate production were F and I; the former described as having *Hippophaë rhamnoides*, *Ligustrum vulgare*, *Ulex europaeus*, and *Hieracium* latter having *Calluna vulgaris*, *Ulex europaeus*, and *Hieracium umbellatum*.

In F *Hippophaë rhamnoides* is a vigorous nitrogen-fixer, and soil sampling at a depth of 10 cm might well bring nodules to the surface. The 5 mm mesh used by Géhu and Ghestem when sieving the samples would allow detached nodules to remain in the incubated samples. The soil would be generally contaminated by the presence of the endophyte from disintegrating root nodules. It would be remarkable if nitrogen-fixing organisms had not been collected in samples from community F.

In I *Ulex europaeus*, the gorse is a legume, which bears the typical root nodules of its family, enabling it to thrive on poor sandy soils. The nodules are larger and more robust than those of *Hippophae*, and it is likely that a 5 mm mesh would prevent them entering the samples for incubation. However the nodules have a finite life, and they gradually disintegrate to liberate nitrates, and endophyte stages into the soil. It would again be surprising if nitrogen-fixers had not been collected in soil from community I.

The evidence for the presence of nitrogen-fixers in any of the other communities is much weaker. Community C is dominated by *Ammophila arenaria* (Marram grass) which has been shown to

contain nitrogen-fixing organisms within the spaces between the clasping leaf - sheaths (HASSOUNA & WAREING 1964). This may account for the moderate record of nitrate produced within soils from this community.

1.3.9 Low nitrate production

In communities A and B the percentage daily production of nitrate was very low. In B it was negative as the level of nitrate at 60 days was less than it was at the start. No data were given for B for the 90 day estimates.

These two communities were very close to the sea, just above high tide. The explanation of the low level of nitrate production may be the poverty of the microflora in the soil. Referring again to the study of dune mycoflora by Brown, she confirms that this part of the succession (described as "open sand" and "foredunes") shows low frequencies of microfungi species, and also low levels of concentration for those species. A short table demonstrates this.

Table 1.10 Percentages of soil plates on which fungi were absent (data from BROWN 1958)

	Open sand	Foredune	Mobile dune	Fixed dune
Alkaline dunes	69	50	10	17
Acid dunes	53	73	17	0

Brown commented that "the mobile foredunes form an exacting habitat for soil organisms ... the sparse fungal colonization is not therefore unexpected... The repeated isolation of

certain species... suggest that they are not accidental invaders but characteristic members of the flora, tolerant of the extreme conditions and perhaps 'escapers' of intenser competition in the older dune soils." (BROWN 1958 p. 653).

#### 1.3.10 Mineralisation ratios

The high results for levels of nitrate in the Hippophaë and Heath communities could be explained by the fact that there was simply more organic matter present in these soils. Géhu and Ghestem measured the "total nitrogen" of all the soils, and published details of three ratios

$$\text{Global mineralisation ratio (GMR)} = \frac{[\text{NH}_3] + [\text{NO}_3]}{N_t} \times 100$$

$$\text{Ammonification ratio (AR)} = \frac{[\text{NH}_3]}{N_t} \times 100$$

$$\text{Nitrification ratio (NR)} = \frac{[\text{NO}_3]}{N_t} \times 100$$

where  $[\text{NH}_3]$  = concentration of nitrogen as ammonia in ppm

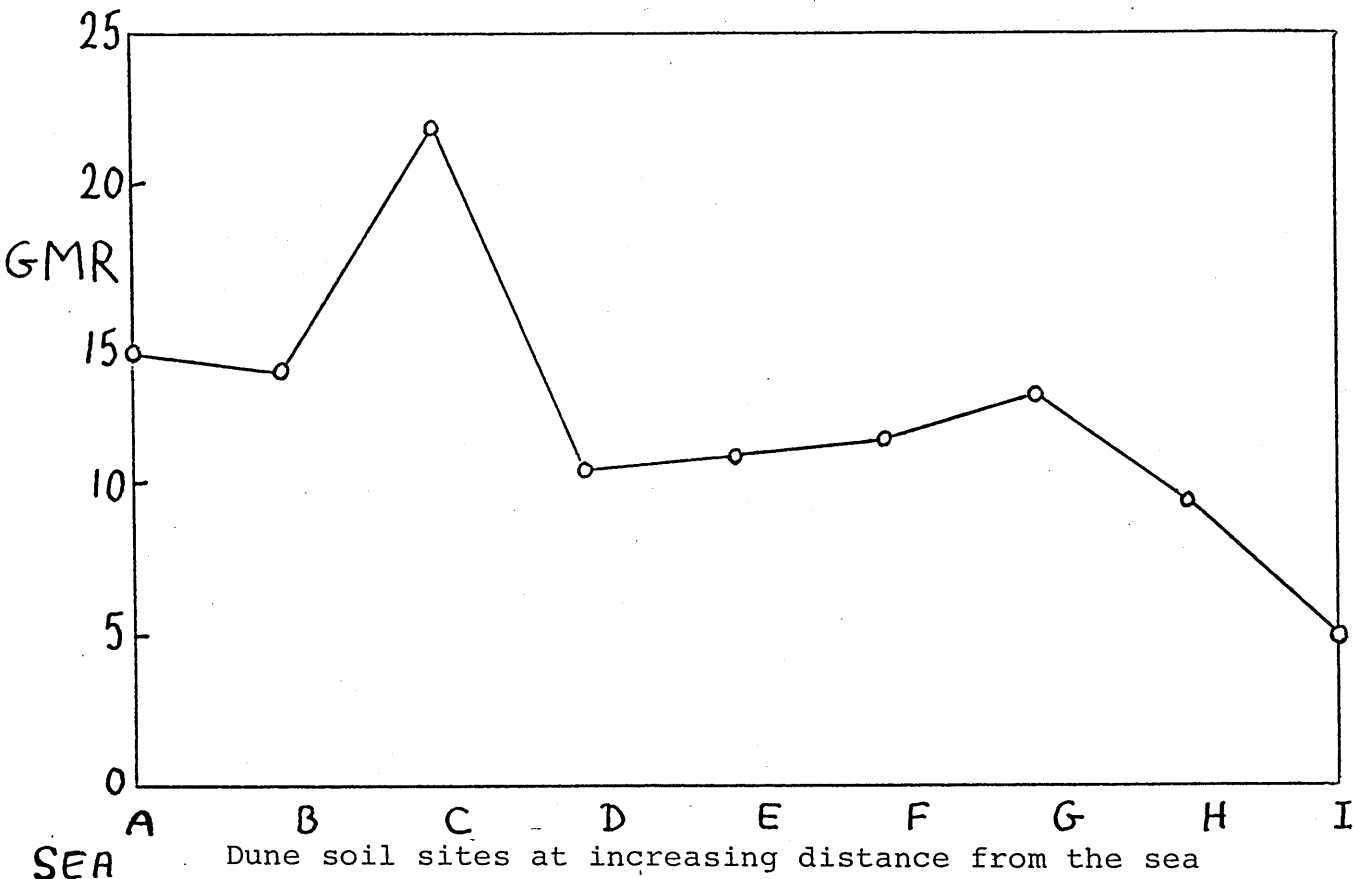
$[\text{NO}_3]$  = concentration of nitrogen as nitrate in ppm

$N_t$  = total nitrogen in ppm

The authors were primarily interested in the possibility that there was a decline in mineralisation with the maturity of the dune communities, and their results confirm this expectation. The value of GMR falls steadily through communities A to I. Only the marram community C is somewhat different from the general trend (Graph 1.22). The decline in mineralisation is presumed to be related to the decrease in soil pH, but the authors bring forward no detailed

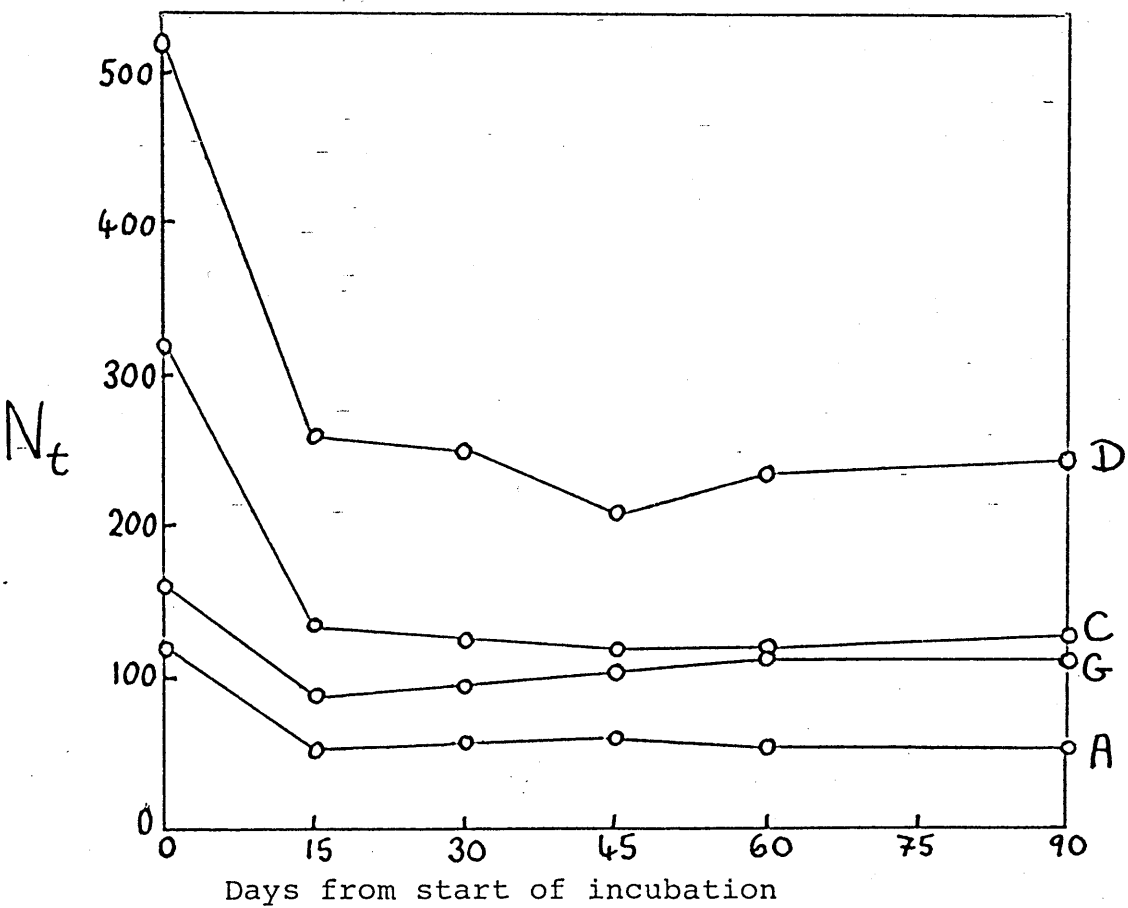
raph 1.22 Global mineralisation ratios at different soil sites.  
(Data from Géhu and Ghestem 1965)

Key: GMR = Global mineralisation ratio at 90 days



raph 1.23 Variation in total nitrogen with time taken in incubation, for several dune soil sites (Data from Géhu and Ghestem 1965)

Key:  $N_t$  = total nitrogen in parts per million of dried soil





evidence in support of this.

It is clear that the Hippophaë and Heath communities no longer stand out as different from the others in respect of the GMR, and neither do they in the AR or NR.

The authors commented on the high value for nitrate in the Hippophaë soils, but only as a means of explaining other situations. "The production of nitrate nitrogen is also somewhat low in the xerosere of the calcareous dunes. There is, however, an important exception in the Hippophaëtum which shows more than 125 ppm of nitrate on the ninetieth day. This fact is sufficient to explain the constant presence of a nitrophil flora beneath the thickets of sea-buckthorn... The other associations of the series produce much less nitrate... Now, these communities do not show the nitrophil vegetation unless some of the sites had been affected by rabbit droppings" (GÉHU and GHESTEM 1965 p.140).

There was no suggestion that the high nitrate levels were due to the large amount of organic matter present. The authors may have considered this implicit in the data on mineralisation ratios. They noted the slight drop in the levels of nitrate after fifteen days in seven of the nine soil types, and concluded that this effect is masked in soil F (the Hippophaë community) by its "considerable biological activity". If this is the case it is surprising that the rate of nitrate accumulation in F is slower after fifteen days than it was before. It might be expected to be the other way around. Soil D showed no fall at all.

The authors were impressed by the fact that "the raw humus of the final successional stage (I) gave, at the end of a dozen

weeks a not inconsiderable release of 'nitric nitrogen'.\* This fact, exceptional enough in an acid medium pH = 5 has however been observed several times, notably by Sittler and Lossaint" (GÉHU & GHESTEM 1965 p. 141).

Implicit in this comment is the idea that an acid medium inhibits the breakdown and mineralisation of organic matter. The authors' results show that the most acid soil produced the second highest level of nitrate. Could this just be due to the large amount of organic matter present, or was it that the nodular endophyte of gorse was accustomed to acid heath conditions and could cope with a pH of 5?

It has been pointed out that even though "nitrate production falls off rapidly below pH 6.0 and generally is negligible below pH 5.0, nitrification can however occur at a soil pH of 4.0 and nitrifying bacteria have been detected in even more acid soils...both *Nitrosomonas* and *Nitrobacter* are obligate aerobes, hence adequate soil aeration is essential..." (RICHARDS 1974 p.151).

#### 1.3.11 Total nitrogen

If it was possible to record the total nitrogen levels in the incubated flasks of soil over a period of 90 days it would be easy to resolve the problem of whether nitrogen-fixation contributed to the process. Although Géhu and Ghestem did not publish data on total nitrogen directly, their bar charts of the levels of nitrate, and ammonia, and the three mineralisation ratios enable one to calculate back to total

\* presumably this means "nitrate".

nitrogen, using three different methods, as a check on accuracy.

$$N_t \text{ (GMR)} = \frac{[\text{NH}_3] + [\text{NO}_3]}{\text{GMR}} \times 100$$

$$N_t \text{ (AR)} = \frac{[\text{NH}_3]}{\text{AR}} \times 100$$

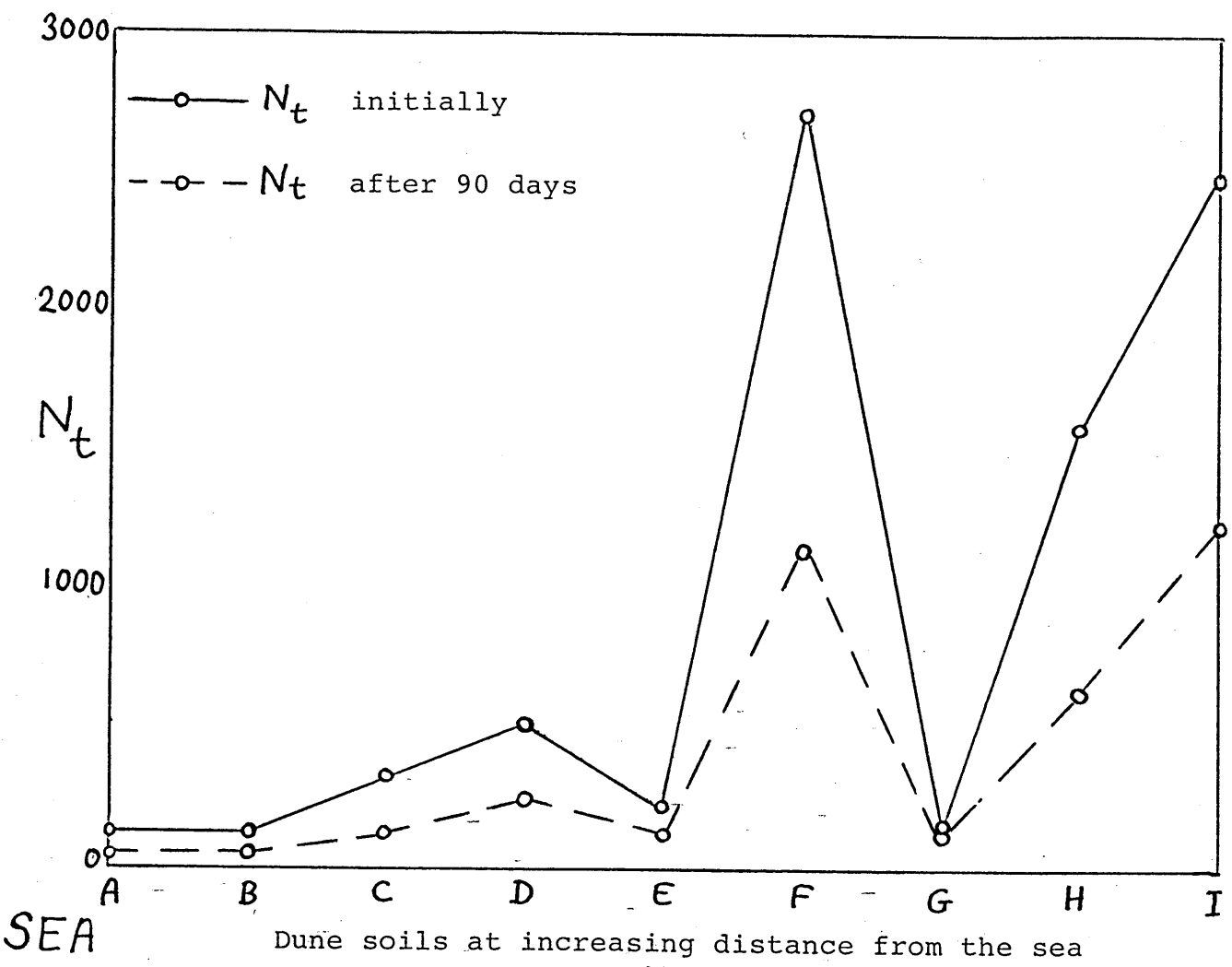
$$N_t \text{ (NR)} = \frac{[\text{NO}_3]}{\text{NR}} \times 100$$

These values were calculated but are not recorded here as a table because of the inherent problems. Firstly the values of  $N_t$  derived from the different ratios do not agree with each other closely, especially near the start of the experiment. Secondly the level of "total nitrogen" falls rapidly in the first fifteen days to about 50% of its original value and afterwards remains constant. (Graph 1.23)

Without the raw data for total nitrogen it is difficult to make any further analysis with confidence. Presumably the authors use the term "total nitrogen" to mean the amount of as yet unconverted nitrogen in the organic matter of the soil, rather than the complete nitrogen content of the flask. It is curious why the level of total nitrogen should fall so dramatically in the first fifteen days, and thereafter should resist further change.

The plot of  $N_t$  (GMR) against the dune communities is instructive. It gives some indication of the relative proportions of nitrogen present, and shows that these do not shift between one community and another during the course of the experiment (Graph 1.24). The two peaks are represented by the *Hippophaë* community (F) and the heath with gorse (I).

raph 1.24 Variation in total nitrogen for several dune soil sites; initially and after 90 days incubation. (data from Géhu and Ghestem 1965).



Key:-  $N_t$  = total nitrogen in parts per million of dried soil

Table 1.11 shows how the drop in total nitrogen across 90 days is very regular, broadly equalling 50% of the initial level.

Table 1.11 Fall in total nitrogen after 90 days incubation.  
(data from GÉHU and GHESTEM 1965)

Soil site	A	B	C	D	E	F	G	H	I
% N <sub>t</sub>	42	54	40	47	62	42	70	40	50

% N<sub>t</sub> Total nitrogen after 90 days, as a percentage of the initial total nitrogen.

The only certain way of establishing whether nitrogen-fixing organisms in dune soil contribute to nitrate levels would be to carry out an acetylene reduction assay. However this study of a French dune system contributed a considerable amount of information on the nature of the mineralisation.

#### 1.4 Effect of nitrogen-fixing colonisers on nitrogen levels in tropical dune soils.

Nitrogen-fixing organisms can have a profound influence on the fertility of the soil, and this is particularly dramatic in the case of very poor sandy soils like those of coastal dune systems. This has been demonstrated clearly in an important study of tropical dune systems in the Cape Verde Islands (DOMMERGUES 1963).

The islands lie about  $15^{\circ}$  N of the equator, some 800 Km west of the coast of Africa. Mobile dunes along the northern coasts of the islands tended to migrate inland under the influence of the prevailing westerly winds. In an attempt to stabilize the dune systems they were extensively afforested with filao (*Casuarina equisetifolia*).

Some years after the afforestation project was started Dommergues took the opportunity to compare the nitrogen levels in the soil of afforested dunes, with a control area which had been left unforested. He determined the nitrogen content of the soil, and also that of the colonizing plant material.

In contrasting the two regions of dune soils it is possible to state that almost all of the nitrogen in the afforested plot is derived from the process of nitrogen fixation. However it is not possible from the Dommergues study to tell what proportion of the current soil nitrogen is derived directly from nitrogen-fixation and what proportion is derived from the breakdown of litter from previous years. It is likely that the nitrogen-fixing capability of the





*Casuarina equisetifolia* - a magnificent specimen  
over five metres high in the hothouses of the  
Edinburgh Botanic Gardens



Bole of  
*Casuarina*





Leaves and cones of Casuarina



Casuarina trees varies with maturity, so a simple calculation based on dividing the present nitrogen content of dune soil by the age of the stand would not yield an accurate estimate of the annual rate of nitrogen-fixation. However it would give a useful average, and the figure obtained by Dommergues was 17.6 kg/ha/yr for soil nitrogen.

The study examined not only soil nitrogen but also the stock of nitrogen in the vegetation growing in the afforested plot. The technique employed was dimensional analysis whereby the nitrogen content of components of an "average tree" was measured. Extrapolation from these measurements to the whole stand were then made, to arrive at the final results, expressed in kilograms of nitrogen per hectare per year.

Dommergues estimated the amount of nitrogen in roots to be 10-20% of that of above-ground components. He accepted the upper figure of 20% and added this to his earlier calculations. He discussed the difference between net fixation and true fixation. It was necessary to subtract from net fixation the figure for nitrogen added from the atmosphere by rainwater, to obtain the true fixation. He considered the problem of losses due to leaching and denitrification but in the absence of any data he limited to himself to stating that these losses certainly exceeded the gains due to nitrogen of atmospheric origin in rainwater. His final figure for fixation overall was 67 kg/ha/yr, and he considered this to be a minimum estimate. It accorded with comparable studies in tropical regions (the Congo Basin, and Ghana) by Greenland, Bartholomew and their associates,

which yielded estimated of 60-170 kg/ha/yr.

The figures below (Table 1.12) are assembled from data published by Dommergues. Given that the study took place in the tropics it is possible to make only general comparisons with European research in this area.

The principal finding appears to be that though the nitrogen-fixing propensity of Casuarina is only modest, its cumulative effect is dramatic in improving the fertility of impoverished soils. The stock of nitrogen in the soil alone increased about four times in the thirteen-year period (from 80 kg/ha in the control to 309 kg/ha in the experimental plot).

If we combine the estimated annual rates of fixation in soil (17.6 kg/ha) and the vegetation mass (67 kg/ha) it yields a figure of 85 kg/ha/yr. This is close to the value of 80 kg/ha in uncolonized soil. This would indicate an annual increment equal to the normal nitrogen level. The cumulative effect would be considerable. It is worth noting again that this kind of conclusion is very inadequately based. A knowledge of the actual annual rates of fixation would be a natural next step from the basic figures provided by Dommergues' study. It is known that nodulation in Hippophaë increases in young plants towards a maximum at three years and from then on it declines, probably because of the nitrogen levels present in the soil (OREMUS 1979). High levels of soil nitrogen inhibit the formation of nodules in Hippophaë and consequently its nitrogen-fixing activity. This need not affect its normal growth because the free soil nitrogen could readily supply its needs. It is not unreasonable to suspect that a similar kind of cycle may exist in the colonization of

dunes by Casuarina.

The conditions on Cape Verde are probably near optimum for nitrogen-fixation in that temperatures are high, sunshine is plentiful, and water is readily available. The levels of nitrogen at 50 ppm in the mineral fraction of the control plot, and 90 ppm in the corresponding part of the experimental afforested plot compare very favourably with European dune soils. The latter might be expected to show values of one-tenth of those recorded on Cape Verde Islands.

Table 1.12 Estimation of nitrogen stock in dune soils of Cape Verde Islands (from DOMMERGUES 1963)

Treatment	Soil horizon	Nitrogen level in ppm	Stock of N in kg/ha
1 Control plot	Mineral 0-10 cm	50	80
2 Afforested plot	A <sub>00</sub> intact litter	8800	137
	A <sub>01</sub> decomposed litter	5600	28
	Mineral 0-10 cm	90	144

### 1.5 Sea buckthorn and impoverished soils

It is not easy to follow the effect of these nitrogen-fixers on the general level of soil fertility because of parallel contributions to soil nitrogen from other sources. In principle, it should be possible to show the effect most easily when the soil in question has no nitrogen of its own, and the only source of nitrogen comes from fixation. Few of the known nitrogen-fixers are capable of colonizing and developing successfully on nitrogen-deficient soils.

Dommergues (1963) showed that *Casuarina* was a most efficient agent of fixation in the impoverished soils of coastal dunes in tropical areas. The analogue of *Casuarina* in the north temperate region is *Hippophaë rhamnoides*, the sea-buckthorn.

This plant is almost entirely confined to impoverished soils, because it has a low competitive ability with regard to light. Once a soil has improved enough to enable the common shrubs and seedling trees to survive and grow successfully they create sufficient shade to inhibit any further development of the sea-buckthorn. Darmer (1948 b) estimated that the light intensity for successful growth in *Hippophaë* was above 3000 lux.

There are two main types of impoverished soil. Coastal dunes originate in the accumulation of sediments, often as offshore bars parallel to the coast, or tapering headlands, formed by marine and river currents. The landward movement of windborne intertidal sand grains builds up dune ridges, which may migrate inland, or extend the coastline seawards. The normal composition of dune soils is limited to quartz particles, with a variable admixture of shell fragments.

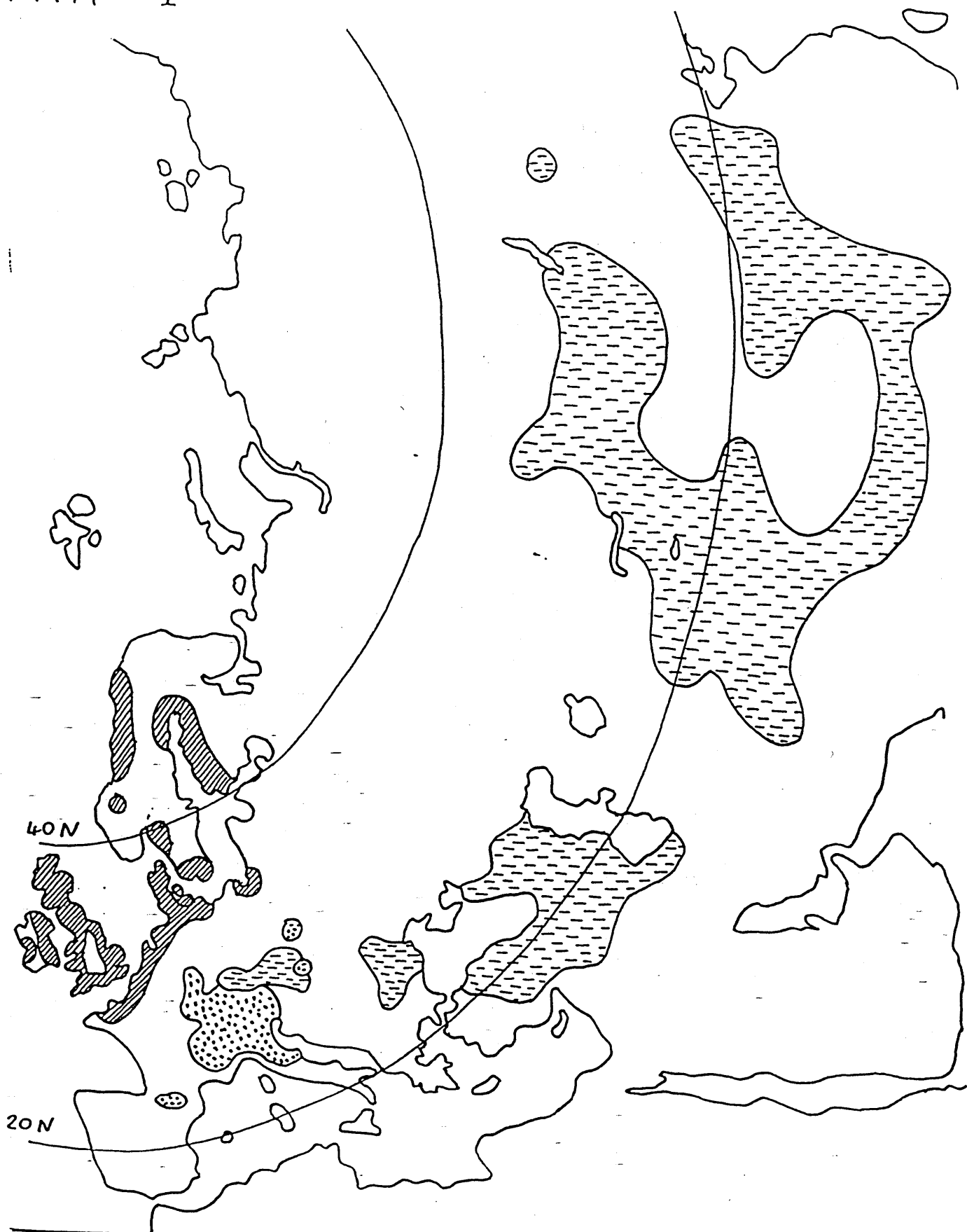
The second soil type originates with the release of sediments from the grip of ice in high mountain regions. The effect of icefields and glaciers is to abrade and grind down the surface of the underlying rock. Eventually a mass of rock flour, gravel and boulders emerges from the glacier snout. Although much of it is carried downstream, a great deal remains within the valley close to the glacier, as a desolation devoid of any plant life for several years. The composition of the soil can vary greatly depending upon the nature of the rock underlying the glacier but it contains no nitrogen in forms accessible to plants.

Studies of the distribution of *Hippophaë rhamnoides* show it to be discontinuous, but confined within a broad band, corresponding to the north temperate zone, and extending from central Asia to the Atlantic coast. (Map 1) Within this zone the plant is concentrated as separate populations in the two types of areas where impoverished soils occur - the coast and the mountains.




The coastal area is limited to NW Europe but the plant distribution remains curiously discontinuous, taking in Norway, the north Baltic, SW Sweden, Denmark, the low countries, and East Anglian Britain. This distribution is shown in Map 2 which incorporates data from the "Atlas of the British Flora", a paper by Pearson and Rogers (1962) and a comprehensive taxonomic study by Arne Rousi which unfortunately excludes from its maps most of the major seabuckthorn colonies of the British Isles (ROUSI 1971, Map 28 p. 222).

*Hippophaë rhamnoides* is represented in all the mountain

## MAP 1

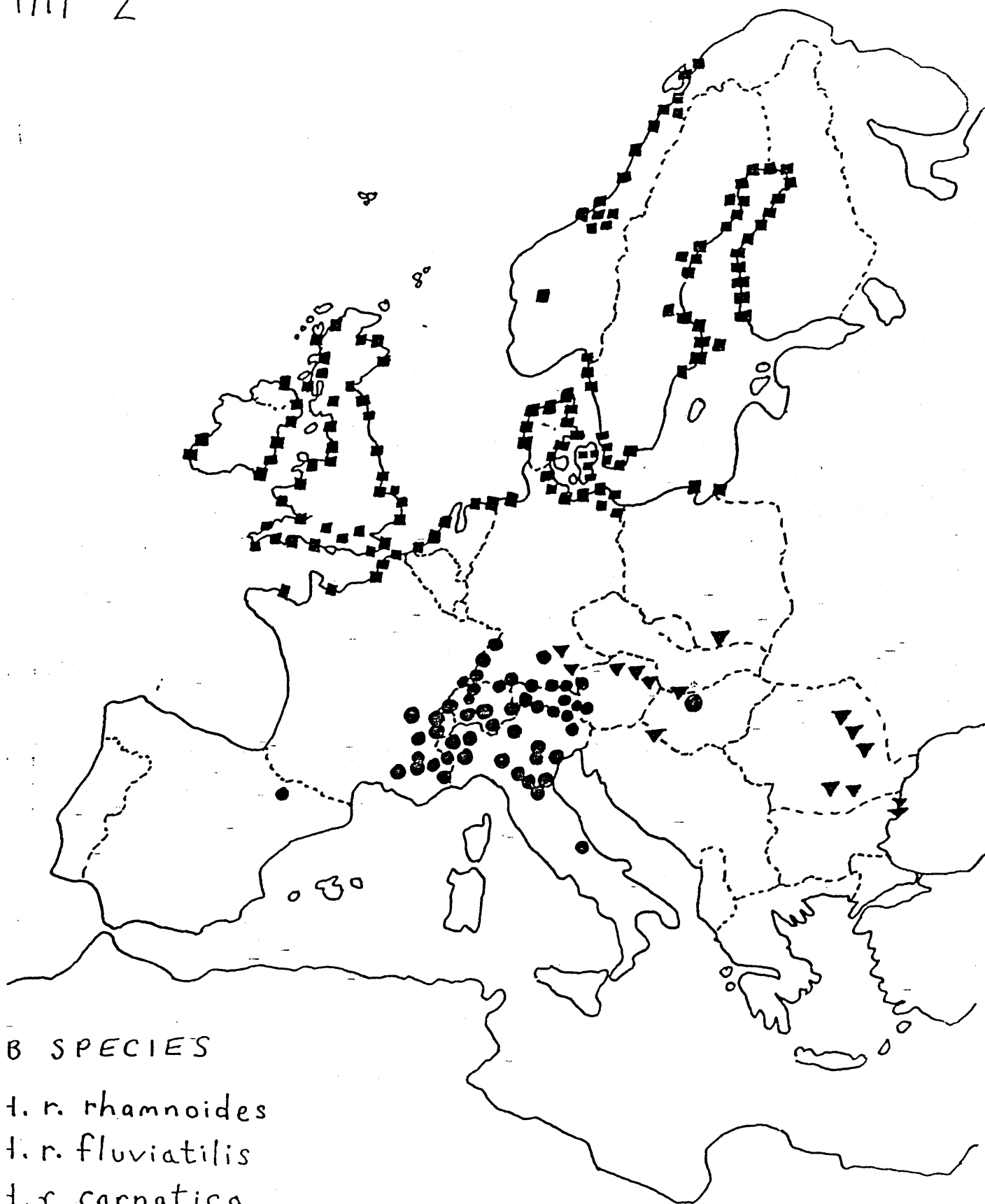


WORLD DISTRIBUTION OF HIPPOPHAË  
RHAMNOIDES L.

 *H. r. rhamnoides*
 *H. r. fluviatilis*
 Asian subspecies

# EUROPEAN DISTRIBUTION OF HIPPOPHAË RHAMNOIDES

1AP 2



B SPECIES

t. r. rhamnoides

t. r. fluviatilis

t. r. carpatICA

chains within the north temperate zone, but there are intriguing discontinuities. Some populations occur in regions which have not been glaciated for centuries (eg. the Italian Apennines, or the Schwarzwald) and there are areas still retaining glaciers which nevertheless have very limited sea-buckthorn populations (eg. the Pyrenees, and the Jotunfell of Norway).

The division of *Hippophaë* distributions into two distinct physical regions has tempted taxonomists to distinguish two separate sub-species. Van Soest (1952) proposed "*H. rh. maritima*" (coastal) and "*H. rh. fluviatilis*" (mountains). It would not be appropriate to spend long examining the variety of different proposals which have been made concerning the taxonomy of *Hippophaë rhamnoides*. It is sufficient for the purposes of this study to note that all the taxonomic authorities have been content to retain *Hippophaë rhamnoides* as a single species with a variable form and distribution. The debate is concerned with the validity and nature of the sub-species which have been proposed.

Much of the research on the influence of sea-buckthorn on soils has been confined to the coastal populations because of the greater convenience in collecting and analysing samples, compared with populations in mountain locations. Consequently comparisons between the findings of different workers are likely to be valid, given the homogeneity of the coastal species. One further possibility needs to be examined before this homogeneity is accepted as a reasonable assumption.

It may be that even if *Hippophaë rhamnoides* is itself a



uniform species, it can enter into a number of different combinations with other species, sufficient to create different ecological or edaphic conditions. Comparisons of results obtained by different workers would be of limited validity unless the basis of these inhomogeneities was fully understood. The detection of these differences lies in the domain of the phytosociologist, and it is clear that competent authorities differ in their conclusions.

Skogen drew attention to the debate on the status of Hippophaë communities among such authorities as Aichinger, Eckmüllner and Gams but he questioned "how far it is justifiable to regard any Hippophaë vegetation as an independent plant community" given the "lack of any real characteristic species" in association with it. (SKOGEN 1972 p. 4)

In a detailed computer analysis of dune-scrub communities in the Delta region of the Netherlands (SLOET VAN OLDRUITENBORGH 1976) Hippophaë was chosen as one of the five axes. The results obtained proved difficult to interpret simply, as sixteen main groups and eighty-five basic types of association were recorded. As the author commented, "it is not easy to understand the present distribution of the scrub species mainly because of the extremely complicated and dynamic environment of the dune landscape" (p. 106). Her observations on scrub complexity confirm those of Skogen, mentioned earlier.

This whole issue of complexity in vegetational analysis and methods of resolving it is the subject of debate among phytosociologists.

The method of successive approximation has been powerfully argued (POORE 1962) as the most economical way of tackling the problem of vegetational variation. The difficulty of assessing homogeneity is discussed at length, and he states that "the worker chooses stands which are as far as possible uniform in general physiognomy, ecology and species composition, as far as these may be determined by inspection. They should also be stands of communities which are typical of the region and occur frequently in it." (p.149) In sampling, Poore discusses the "minimal area" concept, which he regards as very important despite the difficulties of definition. He explains that "the minimal area gives some idea of the size of area to be analysed to give a fairly representative picture of a stand" (p.50).

Although the subjective estimates of plant cover have been criticised on the grounds of inaccuracy Poore has defended the technique because "analytical comparisons are usually confined to differences in presence or absence of species, and to large and consistent differences in abundance. Under these circumstances subjective estimation is not only justified but provides the most economical way of obtaining the necessary data." (p. 52).

In addition Poore has drawn attention to the computer-based studies and their weaknesses. "Very little attention has been paid to the disadvantages of the more rigidly quantitative methods; these may be considered to be excessively abstract and give false security ... the reduction of observations to a table of figures may give a sense of psychological security which unwarranted by the method." (p. 52)

Without going deeper into the controversy it does seem clear that there is no evidence of an unequivocal nature for regarding young Hippophaë scrub as anything other than a homogeneous population within certain broad limits, even though mature plants may form mixed communities with other scrub and herb species.

## 1.6 Release of nitrogen from root-nodules of Hippophaë

Hippophaë improves the nitrogen levels in impoverished soil. Is there evidence that nitrogen compounds are released directly by the root nodules? A Norwegian study (SKOGEN 1972) has provided some interesting results, and the author considered that his "investigations as a whole indicate unequivocally the Hippophaë does release nitrogen from its roots" (p. 90). The following analysis of his results examines the validity of this claim.

Skogen refers to the fact that the nourishing effect of Hippophaë on soils had been recognised qualitatively as long ago as 1774 by the Norwegian geographer Gerh. Schöning (p.84) but the first demonstration that the root nodules accumulate nitrogen came from the classical work of Warming in 1876 (p. 86), who concluded that it was derived from the atmosphere.

Skogen's study was very largely concerned with the phytosociology of a Hippophaë community growing in estuarine conditions within the fjords of Central Norway. This is one of the typically isolated patches of the discontinuous European distribution of the species. The author not only investigated soil nitrogen levels in field conditions in relation to the maturity of sea-buckthorn scrub, but also carried out experiments on the release of nitrogen by the root-nodules.

In the following analysis I have limited myself to the aspects of the study which were directly concerned with the nitrogen economy of Hippophaë - about 10% of the text.

These can be summarised briefly.

- (i) A detailed floristic analysis of the different plant communities was made.
- (ii) The oldest Hippophaë stands were 60-80 years old.
- (iii) Soil samples from all the main communities were analysed for nitrogen and other chemical components.
- (iv) Two series of laboratory experiments were run to investigate the release of nitrogen from the nodules of sea-buckthorn plants.

#### 1.6.1 Diffusion of nitrogen from nodules

In a general discussion of the nitrogen economy of Hippophaë Skogen marshals the evidence for the theory that nitrogen compounds diffuse outwards from the root nodules of the plant to enhance soil nitrogen levels in the vicinity. At the time he carried out this research in 1960-63 the evidence was unconvincing, but as he explains (p. 90) confirmatory studies appeared before the eventual publication of his own research in 1972.

Skogen comments that there was "no conclusive proof that any of this (fixed atmospheric) nitrogen diffuses into the soil around the nodules, where it could be utilized by other plants" (p.86). Although it had been demonstrated convincingly that this diffusion occurred from the nodules of legumes by workers like Virtanen, Laine, and Wilson, the author considered that the investigations of Hippophaë, Alnus, and Myrica, by authorities like Bond, Allen, Virtanen, and Saastomoinen "have yielded inconclusive, and somewhat contradictory results" (p.86)

### 1.6.2 Field investigations

In his floristic investigations Skogen was impressed by the fact that the soil beneath the sea-buckthorn thickets was attractive to nitrophilous plants, and he goes on to list ten such species. It was evident to him that some kind of relationship existed between the level of soil nitrogen, and the presence of sea-buckthorn scrub.

He argued that "if nitrogen escapes from the root nodules, this should lead to a higher nitrogen content of the soil within the dense Hippophaë stands than in similar situations where Hippophaë is lacking (p. 86). This is a simple proposition. Skogen refrained from suggesting what might seem to be the logical extension of the argument - that dune soil nitrogen levels are directly proportional to the maturity of the sea-buckthorn stands.

His data indicate that there is a stage of sea-buckthorn maturity at which soil nitrogen levels reach a maximum. The levels decline in progressively older stands of scrub. Hence low nitrogen in the soil may be due either to the immaturity or the senescence of sea-buckthorn. These findings are derived from a Hippophaë community with individuals of a great age. He notes that "a much lower maximum age is reported from central and western Europe" (p.16). Consequently it is not possible to compare his results with those of other workers.

The data presented by the author as a simple scatter-diagram of community-maturity against soil nitrogen levels (Fig 35 p. 86) without any trend lines. There is a valuable discussion of these results which enables us to eliminate

four of his data points which are non-typical. Here nitrogen levels are low due to the effects of leaching by freshwater currents. This elimination is amply justified by the fact that other chemical data (eg. salinity) also show atypically low values.

Accepting Skogen's restrictions of his data it is possible to re-arrange the presentation to the conventional form, with the independent variable on the x-axis (ie. community-maturity). This yields a graph to which a trend line can be fitted (Graph 1.25) showing the soil nitrogen maximum to occur at community IV B - (*Hippophaë* with *Filipendula ulmaria*, and also *Humulus lupulus*). The latter two are in Skogen's list of ten nitrophilous species.

It would be instructive to discover how soil nitrogen levels vary with the age of the sea-buckthorn scrub. To do this it is necessary to age-date Skogen's communities. Although he discusses the age of the largest specimens (p. 16) over 10.7 m high as between 60 and 80 years there is no information on the possible-ages of the younger communities which he investigated.

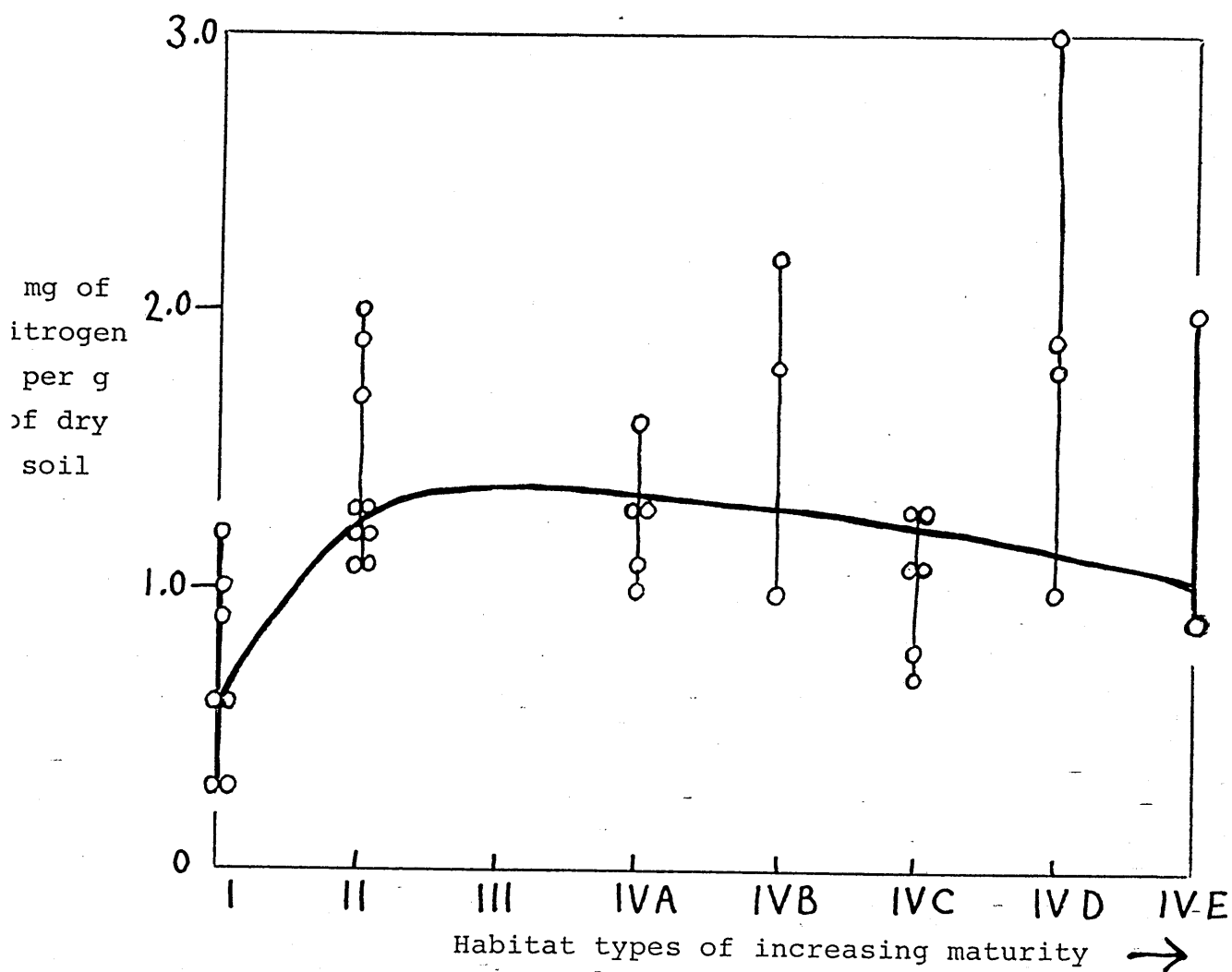
There are three possible sources of error in Skogen's field investigations.

#### (i) Inundation

The Leinöra communities occur on "a group of delta sandbanks situated right at the mouth of the Gaula river, where this flows out into the estuary at Gulosen, a broad arm of the Trondheimsfjord".

"The tidal range at Gulosen is about 2 metres... No part of

raph 1.25 Variation in soil nitrogen within dune habitats of different maturity in a Norwegian dune system. (data from Skogen 1972)





the solid ground lies more than 1 m above sea level ... so that the soil is saturated daily with brackish water" (p. 6).

This region is rather different from the typically coastal sand dunes well above high tide which have been investigated in the countries bordering the North Sea. It is possible that leaching of nitrogen, and also the addition of nitrogen-bearing debris ("quantities of kelp and seaweed debris can often be found ... a metre or so up, in the outermost bushes..." p.7) make it difficult to demonstrate the relation of soil nitrogen to community-maturity.

#### (ii) Litter breakdown

In mature stands of buckthorn one would expect a much denser layer of leaf litter, and this would contribute to the levels of soil nitrogen, notwithstanding any nitrogen-fixation by root nodules. Skogen does not give any direct data on humus, but he claims that "there is no accumulation of humus worthy of mention within the majority of the stands at Leinöra because of the action of tides and floodwaters." (p. 83)

His data in Fig 32 p. 83 show what he calls "a mild humus" with a pH down to about 5.6. Reference has been made earlier to the deposition of marine-derived humus. It is difficult to judge whether litter breakdown complicates the issue or not.

#### (iii) Nodule disintegration

An alternative mechanism to outward diffusion, is the process of nodule disintegration. Skogen suggests, (p.85) that nodules "last for many years". However, a series of studies have shown that they pass relatively quickly through several distinct stages culminating ultimately in degeneration,

necrosis, and death. Andreeva gives a typical account of the sub-cellular changes occurring in Hippophaë nodules grown under laboratory conditions (ANDREEVA et al 1979). It is possible that rates of outward diffusion would also vary during the ageing process. While this process would not affect the validity of Skogen's findings on nitrogen diffusion, they might well explain the levels of soil nitrogen under field conditions.

It has been estimated that 20% of nodules in 7 year old Hippophaë plants were necrotic, and also that many nodules die after three years of age. They are most active in nitrogen-fixation when they are very young. (OREMUS 1979)

Skogen's experiments in the laboratory were designed to see if it were possible to demonstrate the outward diffusion of nitrogen from the root nodules of Hippophaë. He carried out investigations with seedlings and with shoots.

#### 1.6.3 Seedling growth experiments

He germinated Hippophaë seeds and after six weeks of growth exposed the seedlings to an extract of crushed nodules to ensure their infection by the nitrogen-fixing endophyte. He grew the seedlings in three different culture substrates water, vermiculite, and sand, for 110 days, supplying them with the usual minerals except nitrogen. At the end of the experiment the nitrogen content of the substrates was estimated.

The data are presented below in Table 1.13 which is an amended version of Skogen's Table XIV on p.88, as the

calculations of  $P_s$  and  $N_p$  are not given in the original. Fortunately he records the number of plants which died during the course of his experiments, but more to the point is the number which survived, as one would assume this had a bearing on the nitrogen present in the substrate. It is possible to estimate this only indirectly, as he mentions a starting figure of nine seedlings for each water culture, but is not explicit about the other two substrates. However it is assumed that this was nine also, hence  $P_s = 9 - P_d$ .

Again Skogen records the extent of root nodulation qualitatively as "good, sparse, or poor". Had he counted the actual number and recorded this it might have been possible to show that the nitrogen increase was directly proportional to the number of nodules present. This would have added strength to the evidence for the theory of diffusion of nodular nitrogen. However tantalising this omission appears, it must be remembered that the study was primarily phytosociological, and the experiments in the laboratory were something of a side issue.

Table 1.13 Nitrogen-free culture of Hippophaë seedlings (data after Skogen 1972)

culture substrates										
Replicate number	water				vermic.		sand			
	1	2	3	4	5	6	7	8	9	
P <sub>d</sub>	1	2	1	0	2	0	5	8	0	
P <sub>s</sub>	8	7	8	9	7	9	4	1	9	
N <sub>t</sub>	5.7	5.0	6.1	9.5	71.5	83.8	20.1	8.9	72.8	
N <sub>p</sub>	0.71	0.71	0.76	1.06	10.21	9.31	5.03	8.9	8.09	
R	G	S	G	G	G	G	P	P	G	

KEY:

P<sub>d</sub> = number of plants which died

P<sub>s</sub> = number of plants surviving to the end

N<sub>t</sub> = total nitrogen increase in the

substrate - mg

$$N_p = \text{nitrogen increase per plant} = \frac{N_t}{P_s}$$

R = root nodule status

G = good    P = poor    S = sparse

Vermic. = vermiculite

#### 1.6.4 Young shoot growth experiments

In a parallel set of experiments Skogen made use of "young shoots" actually shoot/root systems with nodules attached (up to 2 cm diameter). These were grown in the three substrates, 4 samples in water, 3 in vermiculite, and 1 in sand for a period of 22 weeks (154 days) and cultured with a nitrogen-free mineral nutrient solution. At the end of the experiment the nitrogen content of the substrate was estimated.

The data are given in Table 1.14 based on Table XV on p. 89 of the original paper. No information was available on the state of nodulation.

Table 1.14 Nitrogen-free culture of shoot/root systems of *Hippophaë* plants (data after Skogen 1972)

	culture substrates							
	water				vermic.			sand
Replicate number	10	11	12	13	14	15	16	17
Survival - weeks	22	22	7	5	22	10	9	22
$N_t$	55.9	61.2	22.1	19.1	85.2	60.1	44.4	90.0

(Survival = the number of weeks the plants survived in the 22-week experiment)

#### 1.6.5 Significance of results

Skogen commented on the very low values for nitrogen in the water cultures, suggesting that "this may have been due to the relatively restricted volume of culture solution and the

purified nutrients therein, leading to a deficiency of some trace elements which are important in nitrogen fixation".

(p. 90) What was surprising was the close similarity between values of  $N_p$  for seedlings with "good" nodulation (1,3) and that for those with "sparse" nodulation (2).

The values of  $N_p$  obtained for the seedlings in vermiculite and sand were at least within one order of magnitude (ranging from 5-10 mg.) It is interesting that the value of  $N_p$  for sample 8 with "poor" nodulation, is so close to that for sample 9 with "good" nodulation.

In the experiments with shoots, four of the eight plants died before the end of the experiment. The water cultures of shoots showed values of  $N_t$  lower than those for the other substrates. This agreed with the findings for seedlings. In the surviving four plants the data for  $N_t$  were in reasonable agreement (56 and 61 for water, and 85 and 90 for other substrates).

These experiments indicate the possibility of nitrogen release from *Hippophaë* root nodules, but their main weakness is lack of adequate controls, and the small number of replicates. It is important to determine whether nitrogen is released by non-nodulated roots alone. A control of this kind is particularly essential when the experiments reveal as much nitrogen released from poorly nodulated seedlings, as from well-nodulated ones.

Skogen commented that "although the culture series are limited in number ... the investigations as a whole indicate unequivocally that *Hippophaë* does release nitrogen from its

8<sup>c</sup>

roots" (p. 90). This sidesteps the main issue, which is whether the evidence shows that the nodules release nitrogen. All his earlier discussion was concerned with this point, not just roots in general.

A larger number of replicates is necessary to give some idea of the extent of variation in nitrogen release, and its probable relation to the nodule number present in each sample. This evidence, together with an examination of the variation in nitrogen release with nodule age might provide the kind of experimental proofs which could be considered unequivocal.

Skogen speculates about the nature of the nitrogen released; "it would be but a short step to assume that it is an organic compound which after first having been broken down by micro-organisms, can be absorbed and utilized by the higher plants ..." (p. 90). The experiments estimated only total nitrogen in mg N per g of dry soil probably by some variant of the Kjeldahl method although this is not specified. (p. 4).

In conclusion, Skogen refers to some of the more detailed physiological studies made in the sixties, prior to the publication of his own work in 1972. "These results have subsequently been confirmed by Stewart and Pearson (1967) for *Hippophaë* and by Daly (1966) for *Alnus rugosa*."

This study was an important stage in developing understanding of the ecological effects of *Hippophaë* colonization because it linked together laboratory experiments with the observation of field conditions. It is difficult to accept, however, the author's conclusions on nodular diffusion. Neither is it easy to see how the work of Stewart and Pearson (1967)

confirmed his results as they did no experiments on this particular aspect of the nitrogen economy. Their study was principally concerned with the accumulation of total nitrogen within plants and nodules of *Hippophaë* of a range of ages.

A closer examination of the findings of Stewart and Pearson now follows. Their research prompted a series of questions which were finally resolved by the work of Oremus (discussed in Sections 7,8 and 9.)

#### 1.6.6 Harvest methods with *Hippophaë* scrub

Four sites were selected at Gibraltar Point, Lincolnshire, a stretch of dunes covered by a vigorous and abundant population of *Hippophaë*. Sites I to IV were in increasing order of age. One metre squares were selected and the whole was cropped for the total plant, including roots and nodules. The total nitrogen content was estimated, together with the approximate age based on the annual rings on the leader stems.

The initial results were presented in their Table 1 (p. 351). I have included two other components and given this as Table 1.15 with a key below.

The total plant weight increase over a relatively short space of time is remarkable and the authors suggest that this is due "not only to increase in height of the bushes but also to increase in density of the stands due to the extensive vegetative propagation of the underground system" (p. 351).



Table 1.15 Growth of Hippophaë scrub of different ages  
(data from STEWART & PEARSON 1967)

SITE	A	P	N	$\frac{N}{P} \times 100$	n	$\frac{N}{n} \times 1000$	log P	log N
I	3	1053	2.5	0.24	98	26	3.02	0.40
II	11	1997	39.0	1.95	370	105	3.30	1.60
III	13	4317	64.2	1.49	313	205	3.64	1.80
IV	16	1329	2.7	0.02	22	123	4.14	0.43

KEY:

A = mean age of plants in years

P = total plant weight in grams

N = total nodule weight in grams

$\frac{N}{P} \times 100$  = nodule weight as a percentage of total plant weight

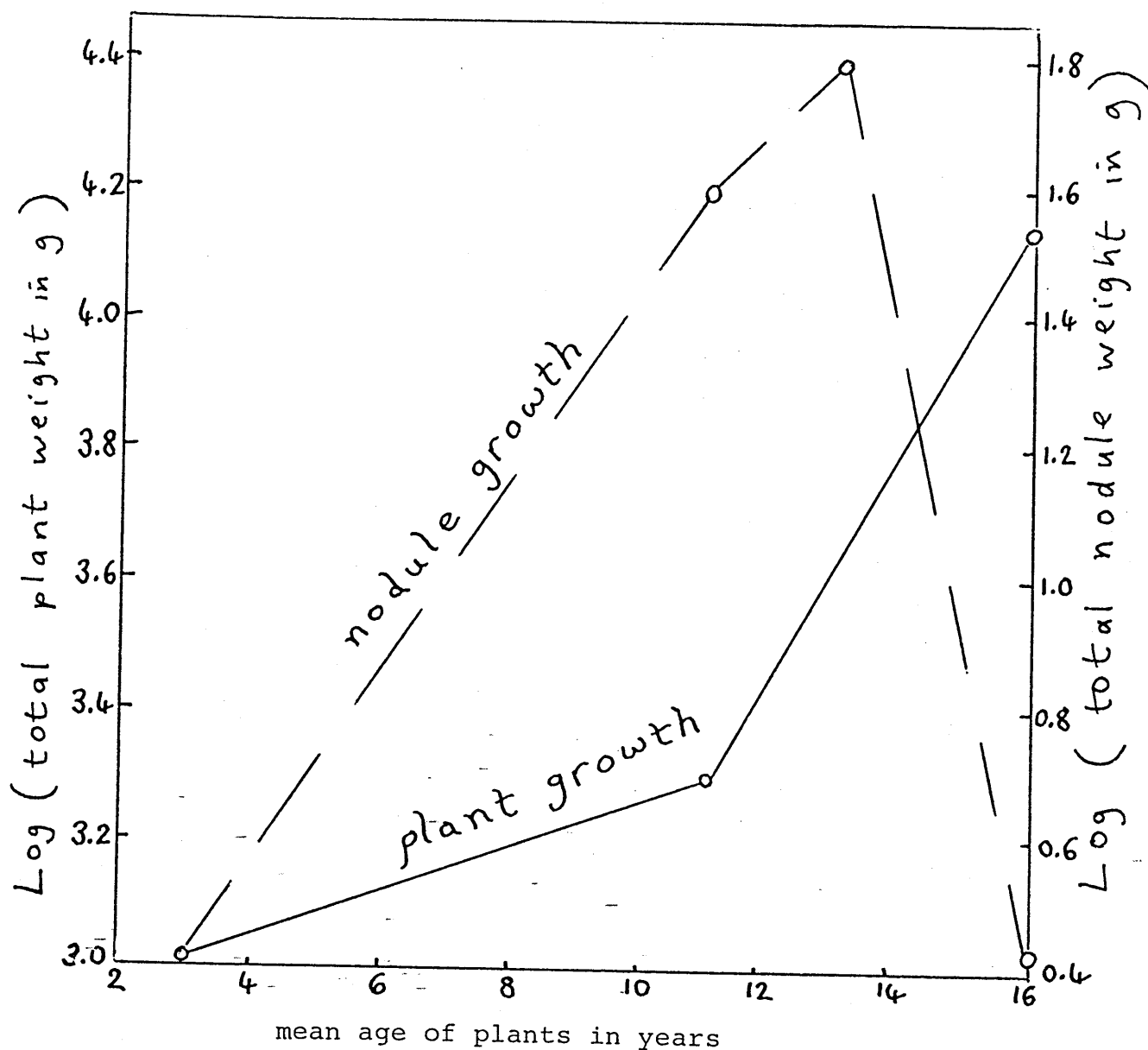
n = number of nodules

$\frac{N}{n} \times 1000$  = mean weight per nodule in milligrams

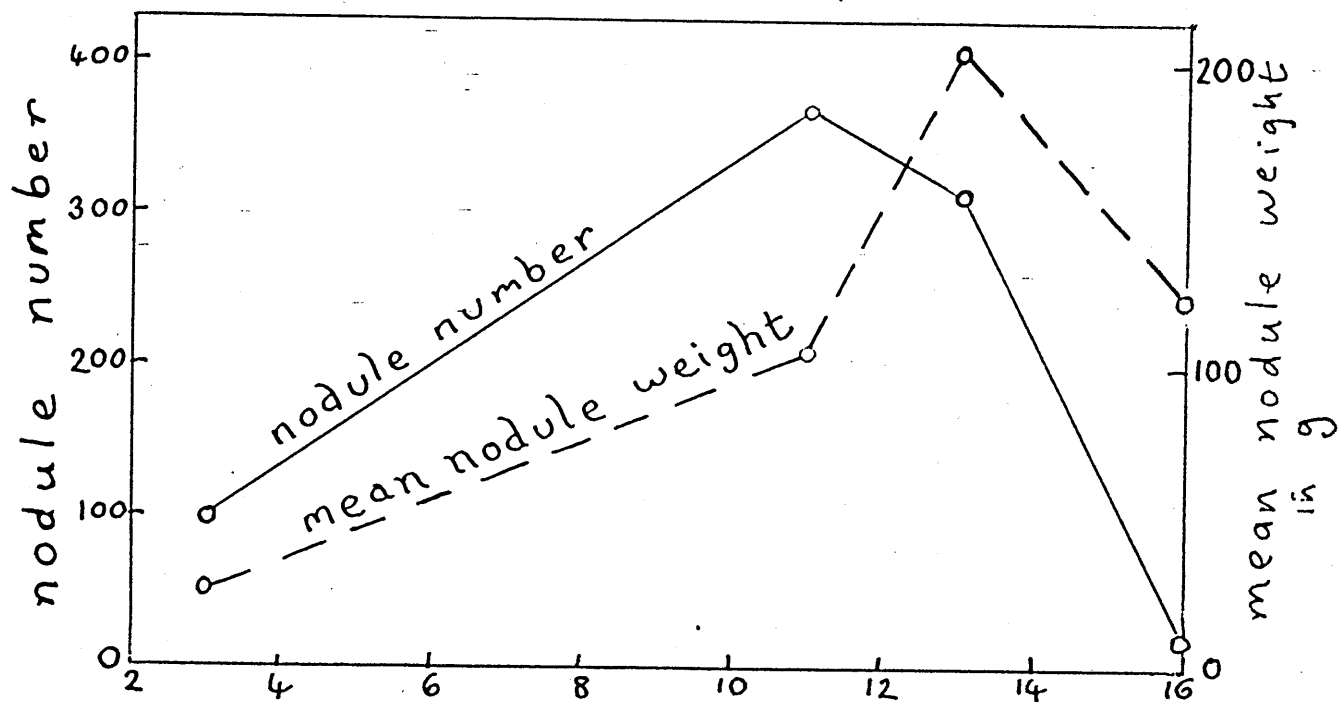
Graph 1.26 shows that while the increase in P is rapid it is not logarithmic, while by contrast the increase in nodule weight, N is faster, and is logarithmic initially, though a decrease takes place later. As graph 1.27 shows most of the results echo this picture of nodulation increasing to a maximum at 11 or 13 years and thereafter declining. The authors made some suggestions in explanation of this phenomenon. Possibly "the bushes at Site I were not fully nodulated" hence the low value. (p.352) The low values at Sites III and IV "may be related to the higher levels of combined nitrogen which occur on moving up the dune system".

However the authors failed to distinguish between live and dead nodules and this is important in the life history of

raph 1.26 Growth in Hippophaë scrub of different ages (data from Stewart and Pearson 1967)



aph 1.27 Variation in Hippophaë nodules with age of plants. (data from Stewart and Pearson 1967)



Hippophaë as Oremus was to show later.

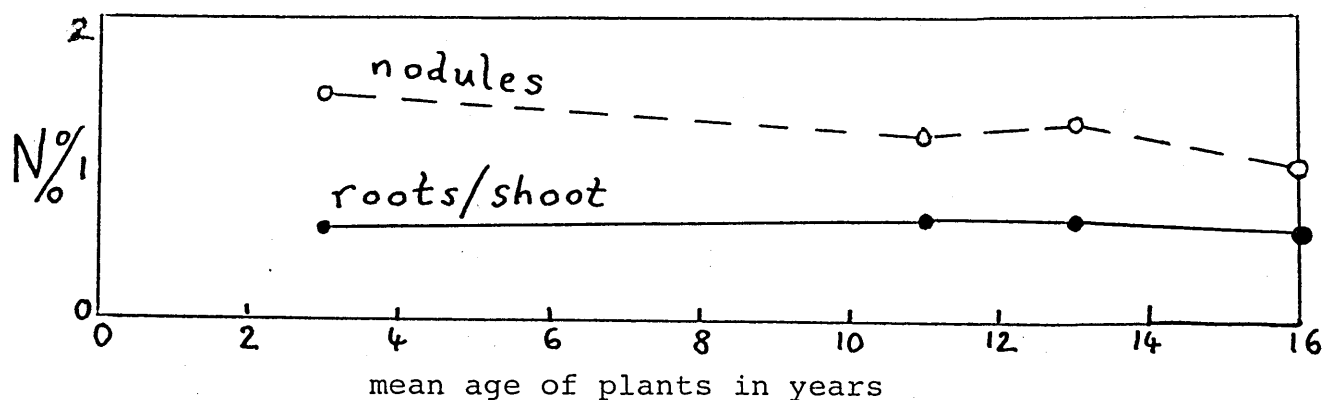
The total nitrogen content of the plant root and shoot material was measured together with the total nitrogen of the nodules, and it was noted that "the percentage nitrogen contents of the nodules are approximately twice as great as that of the root plus shoot material suggesting that the nodules are fixing nitrogen" (p. 352).

We know from other evidence that the nodules fix nitrogen, but this is not a reasonable deduction from the total nitrogen data. There is a relatively high proportion of young nodules among the total number, and young tissue tends to be more proteinaceous, and consequently higher in nitrogen. A significant part of the whole plant is composed of woody tissue which has a lower nitrogen content. Graph 1.28 shows how the mean percentage nitrogen content of roots/shoots and nodules varies with the age of the scrub. (data from Table 2 p. 352) The changes are so slight they do not seem to be significant.

A more useful figure given by the authors is that of whole plant nitrogen per square metre. When these are plotted in Graph 1.29 it is clear that the plant is accumulating nitrogen and the amount increases exponentially. The nodules contain a much smaller amount of nitrogen and this is seen to reach a maximum at thirteen years and then decline. The lower level is due probably to the dynamic state of the nodules which fix nitrogen and then pass it on to the plant.

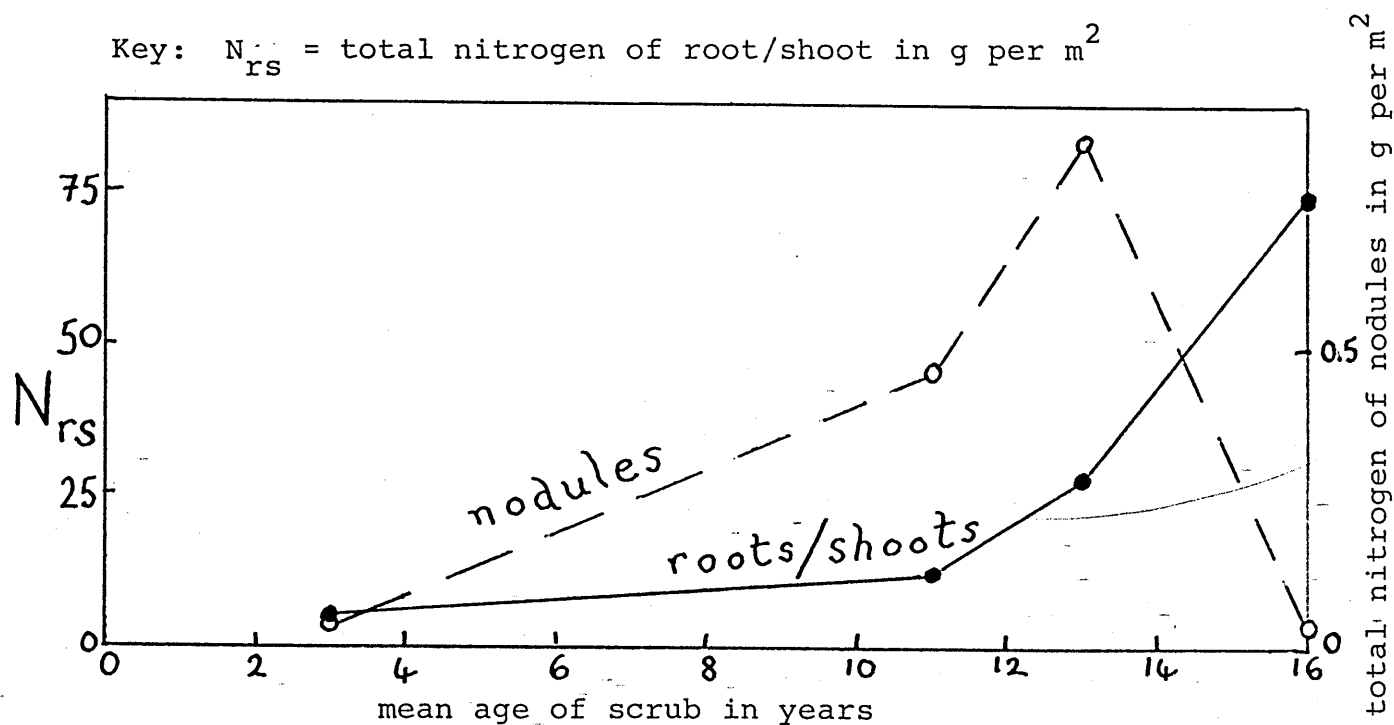
Graph 1.28 Variation in nitrogen content of Hippophaë plants with age. 94  
(data from Stewart and Pearson 1967)

Key: N% = mean percentage nitrogen content



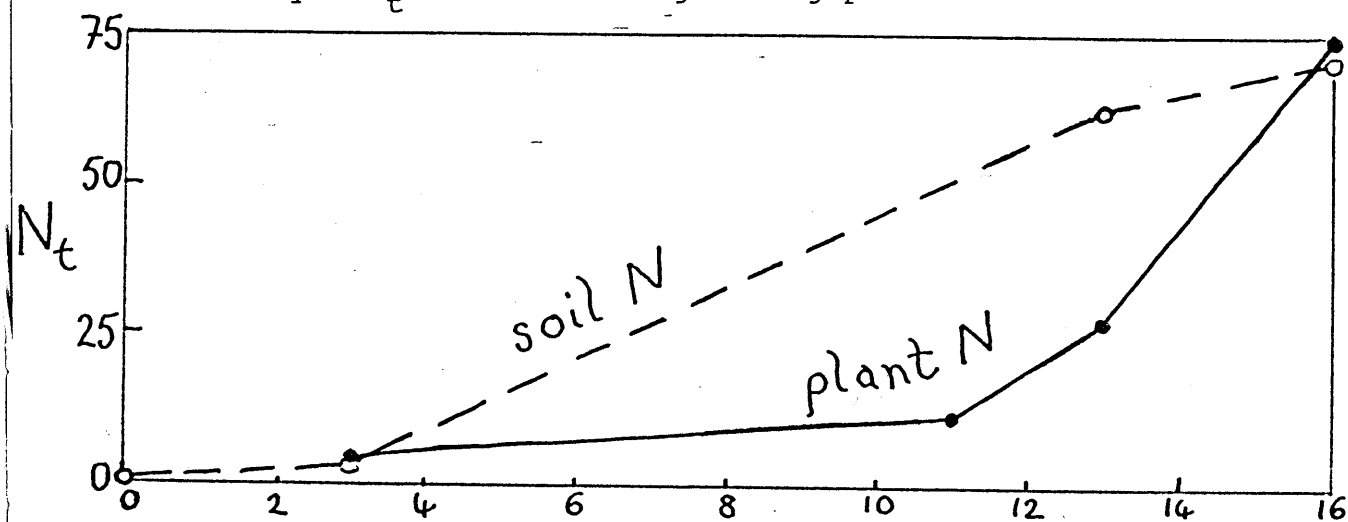
Graph 1.29 Variation in whole plant nitrogen content per square metre with age of Hippophaë scrub (data from Stewart and Pearson 1967)

Key:  $N_{rs}$  = total nitrogen of root/shoot in g per m<sup>2</sup>



Graph 1.30 Variation in soil Nitrogen and whole plant Nitrogen with age of Hippophaë scrub (data from Stewart and Pearson 1967)

Key:  $N_t$  = total nitrogen in g per m<sup>2</sup>



### 1.6.7 Soil analyses

The results of the soil analyses obtained by Stewart and Pearson are of particular interest in connection with the life cycle of the root nodules. In Table 3 (p.353) the value of total N per m<sup>2</sup> of soil is given. This is plotted on Graph 1.30 together with the value for the plant itself. The results are intriguing in that at Site I (3 year stand) the values for soil and plant are almost identical. After this period the soil nitrogen increased rapidly and then slowed while the plant nitrogen increased slowly at first and then more quickly until by 16 year stand the two sets of values were very close again.

The authors commented that "the large relative increase in plant: soil nitrogen at Site IV perhaps implies that assimilation of soil nitrogen by the plant is occurring on an appreciable scale" (p. 353). Examining Graph 1.30 this would suggest that for some reason soil nitrogen levels increase rapidly after three years, but eventually the rate of assimilation by plants increases to meet this. It is possible to explain this phenomenon by the gradual disintegration of root nodules after 3 years, and the increasing size of the plants would enable them to assimilate an increasing amount of soil nitrogen from the nodule remains.

The picture is clarified when the data are considered incrementally; that is, the annual increment in grams per square metre per year. Table 1.16 contains data from Tables 2 and 3 from Stewart and Pearson (p. 352-3) with the incremental elements added.

Table 1.16 Nitrogen increments in Hippophaë growth.

(data from Stewart &amp; Pearson 1967)

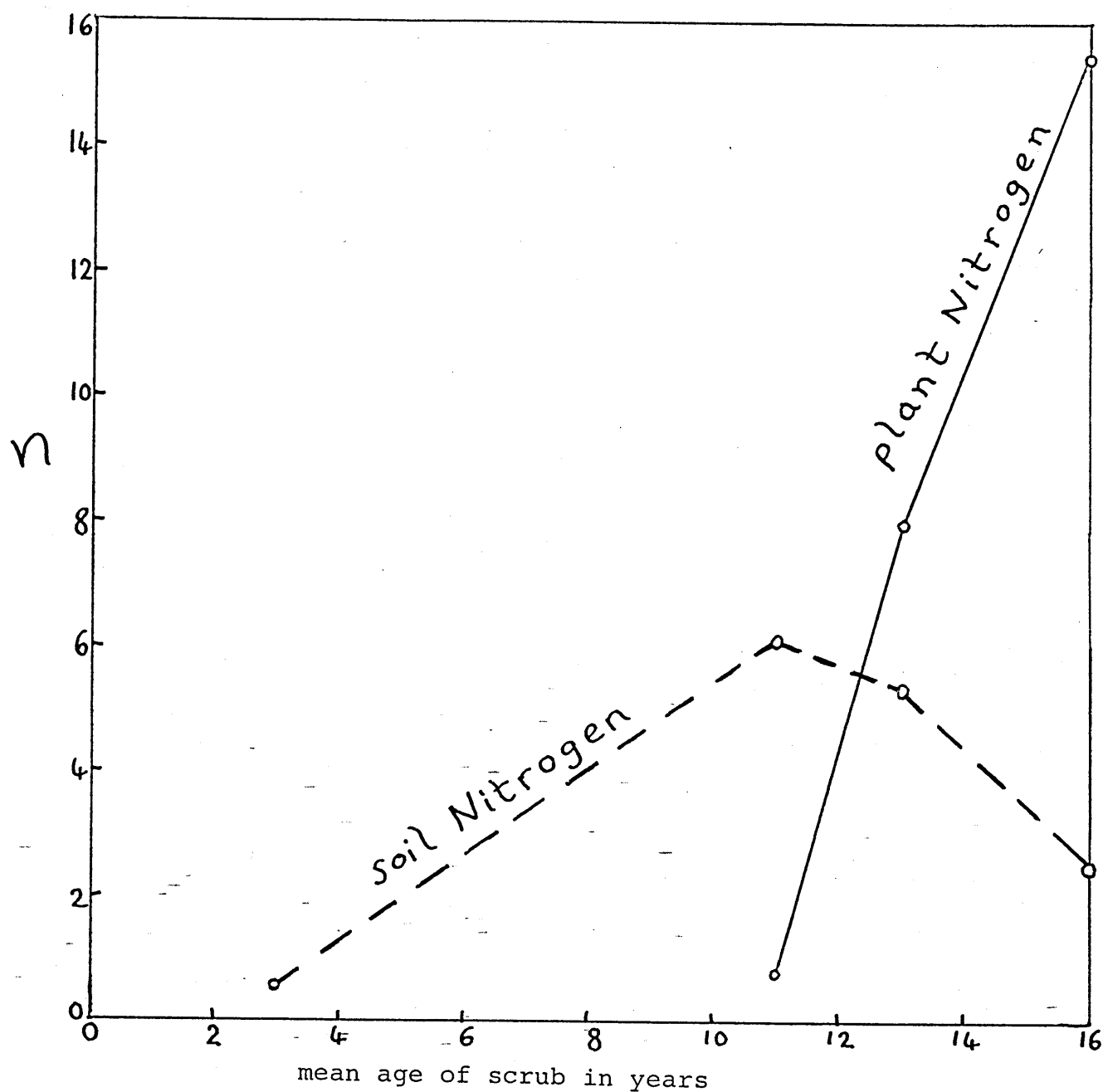
Age of stand	Age increment	Soil N total	Soil N increment	Plant N total	Plant N increment
0	-	1.96	-	-	-
3	3	3.68	1.72	6.45	-
11	8	52.54	48.86	13.00	6.55
13	2	63.28	10.74	28.91	15.91
16	3	70.62	7.34	75.23	46.32

Age	soil increment $\text{g/m}^2/\text{yr}$	plant increment $\text{g/m}^2/\text{yr}$
3	0.57	-
11	6.11	0.8
13	5.37	7.96
16	2.45	15.44

These results are plotted in Graph 1.31 and it is evident that the plant accumulates nitrogen at an increasing rate as it grows older, which is what one would expect, while the soil shows a similar picture to that of the root nodules in earlier graphs.

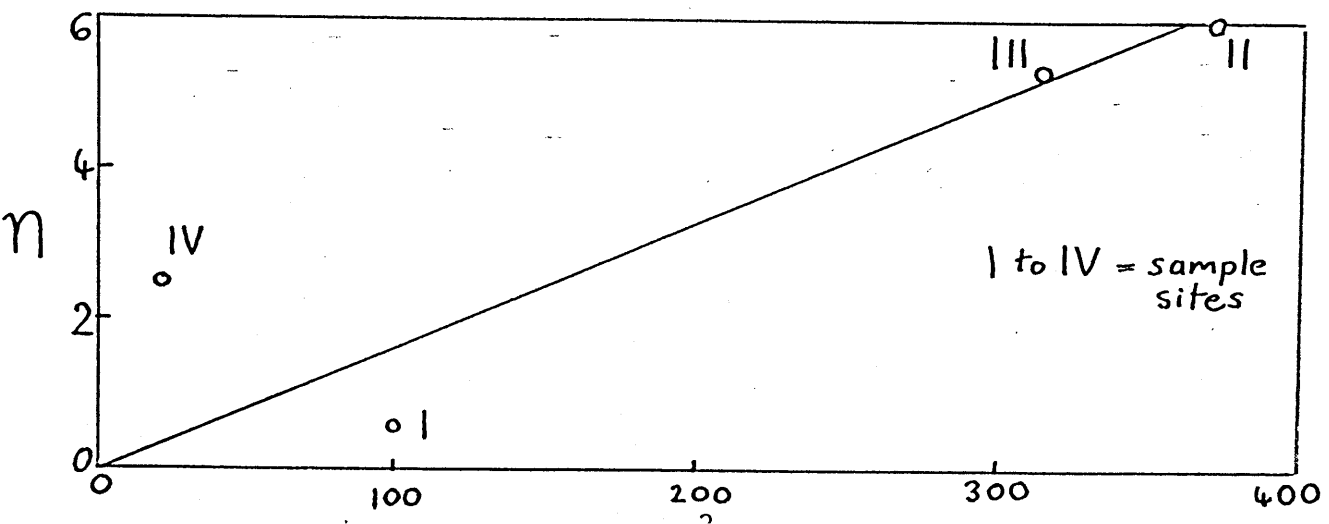
The data appear to point to the conclusion that the annual rate of accumulation of nitrogen by the soil is directly proportional to the extent of nodulation. This is shown on Graph 1.32 where the annual increment of soil nitrogen ( $\text{g/m}^2$ ) is plotted against the nodule number. Although there are only four data points the relationship is clear. Site 1 shows a lower level of soil nitrogen than might be predicted, probably because most of the nodules are young and have not

Key: n = annual increment of Nitrogen in g per m<sup>2</sup> soil



Graph 1.32 Comparison of rate of accumulation of Nitrogen with nodule counts (data from Stewart and Pearson 1967)

Key: n = annual increment of soil Nitrogen in g per m<sup>2</sup> soil



yet begun to disintegrate. By contrast Site IV indicates a higher level than expected possibly because at this late stage most of the nodules are disintegrated, and the nitrogen is still being leached out and absorbed by the plants.

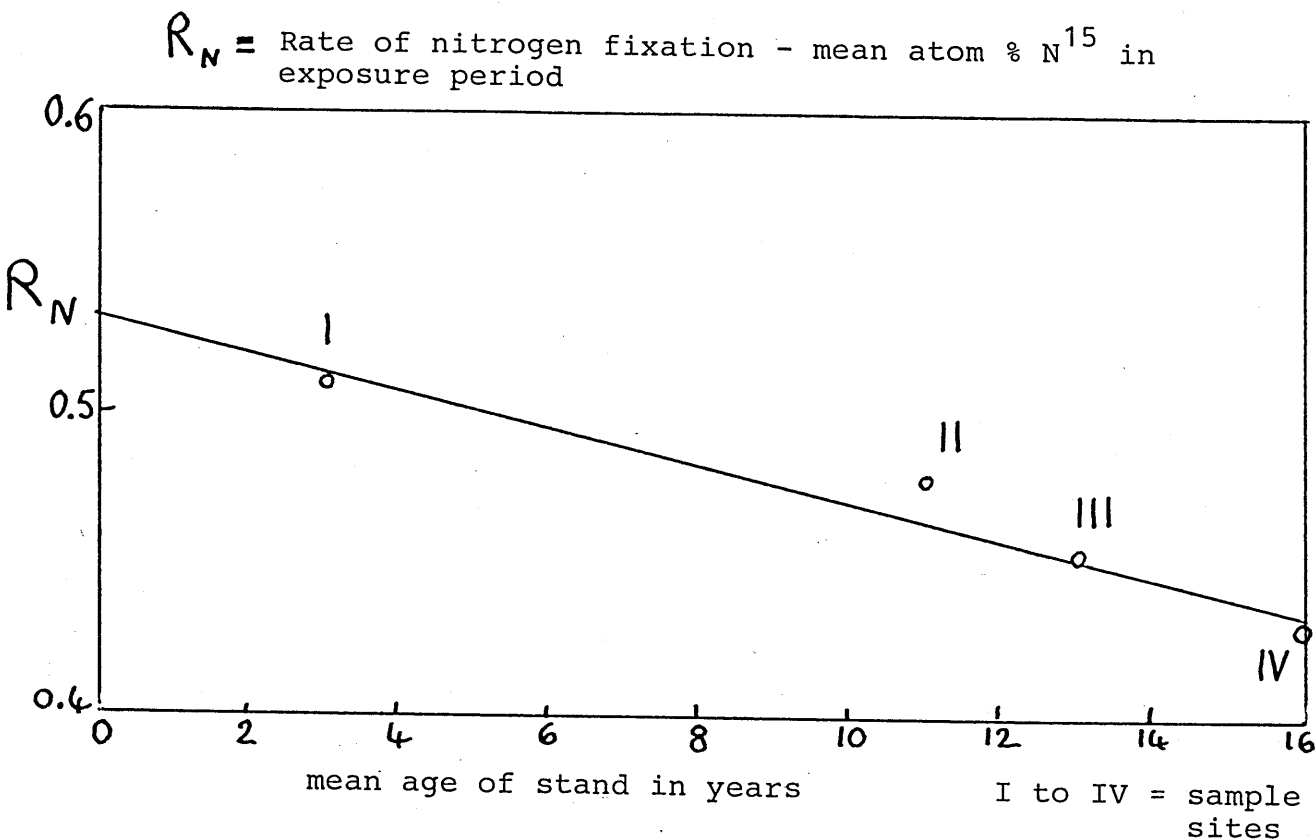
#### 1.6.8 Nitrogen fixation

Seeking greater detail Stewart and Pearson detached roots with nodules attached, and measured the rate of nitrogen fixation by the use of radio-active  $N^{15}$  as a label. The results were presented in Table 5 (p. 354) and one of these parameters is given in Graph 1.33, which shows clearly that the rate of fixation by nodules declines with the age of the Hippophaë stand. The authors considered that "this is probably due to the nodules being perennial structures and thus the older ones contain a higher proportion of wood and cork which contributes nothing to fixation" (p. 354).

It is also possible that this observed effect is due to the presence of a higher proportion of necrotic nodules in the samples taken from older stands. Without a lot more information it would be difficult to be sure about this. In discussing their methods, the authors explained that they excavated 12 cm lengths of Hippophaë root "bearing at least eight typical nodule clusters" (p. 350) and subjected them to  $N^{15}$  exposure. As they had not used any other kind of conscious selection process on their material it does seem possible that more necrotic nodules would be present in the roots from site IV, bearing in mind the discoveries of Oremus (1979) which would not, of course, have been available to Stewart and Pearson in the late sixties.



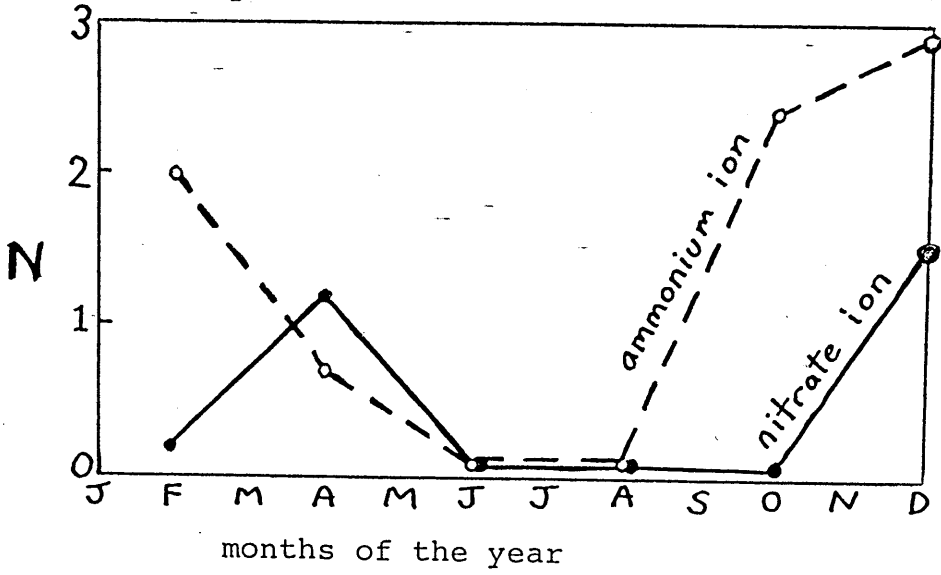
Graph 1.33 Rate of Nitrogen-fixation in Hippophaë stands of different ages. (data from Stewart and Pearson 1967)



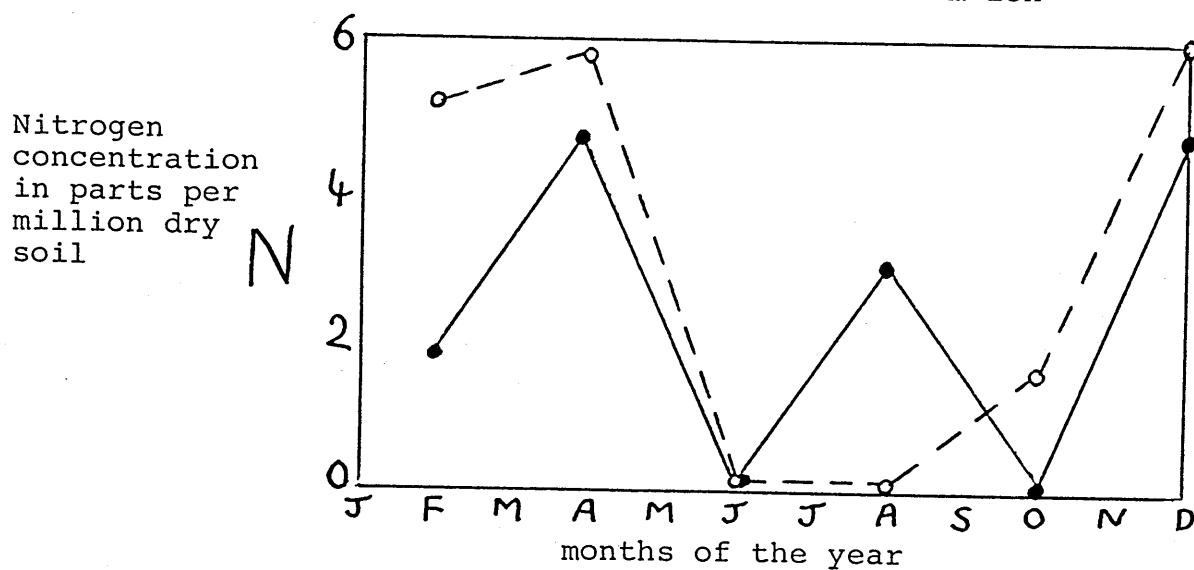
Seasonal variation in soil nitrate at four different sites - Graphs 1.34 to 1.37 (data from Stewart and Pearson 1967)

Graph 1.34 At site 1

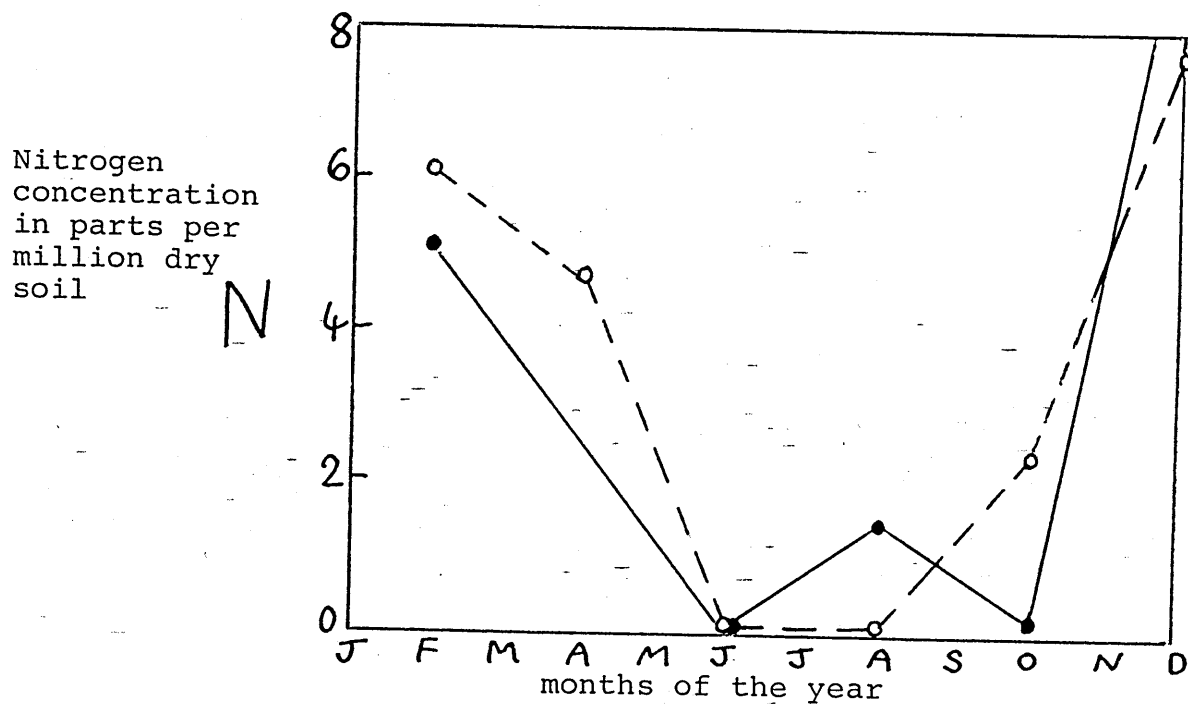
Key: N = Nitrogen concentration in parts per million dry soil



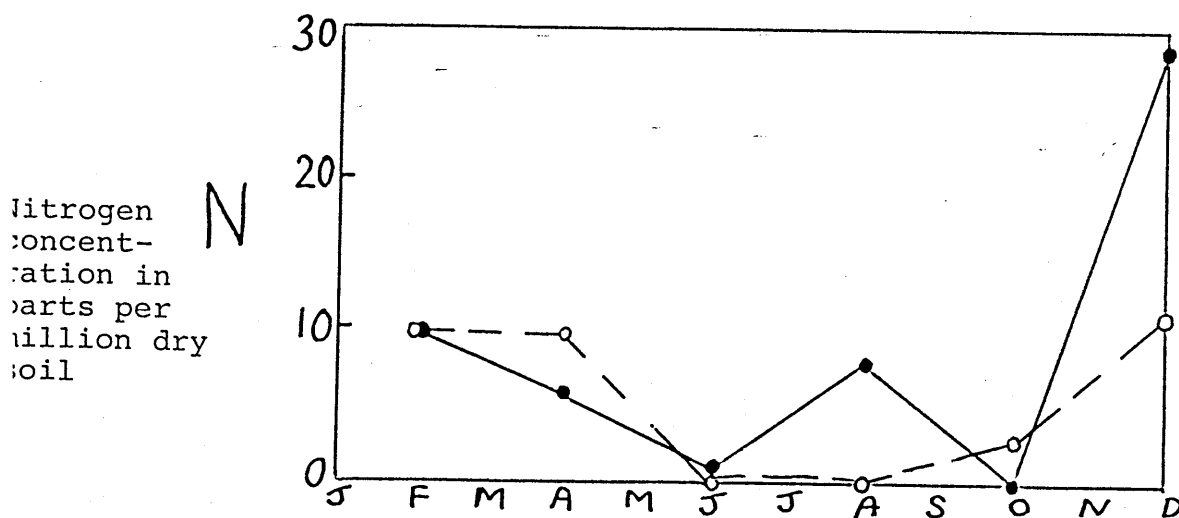
Graph 1.35 Site II Key —●— nitrate ion  
—○— ammonium ion



Graph 1.36 Site III



Graph 1.37 Site IV

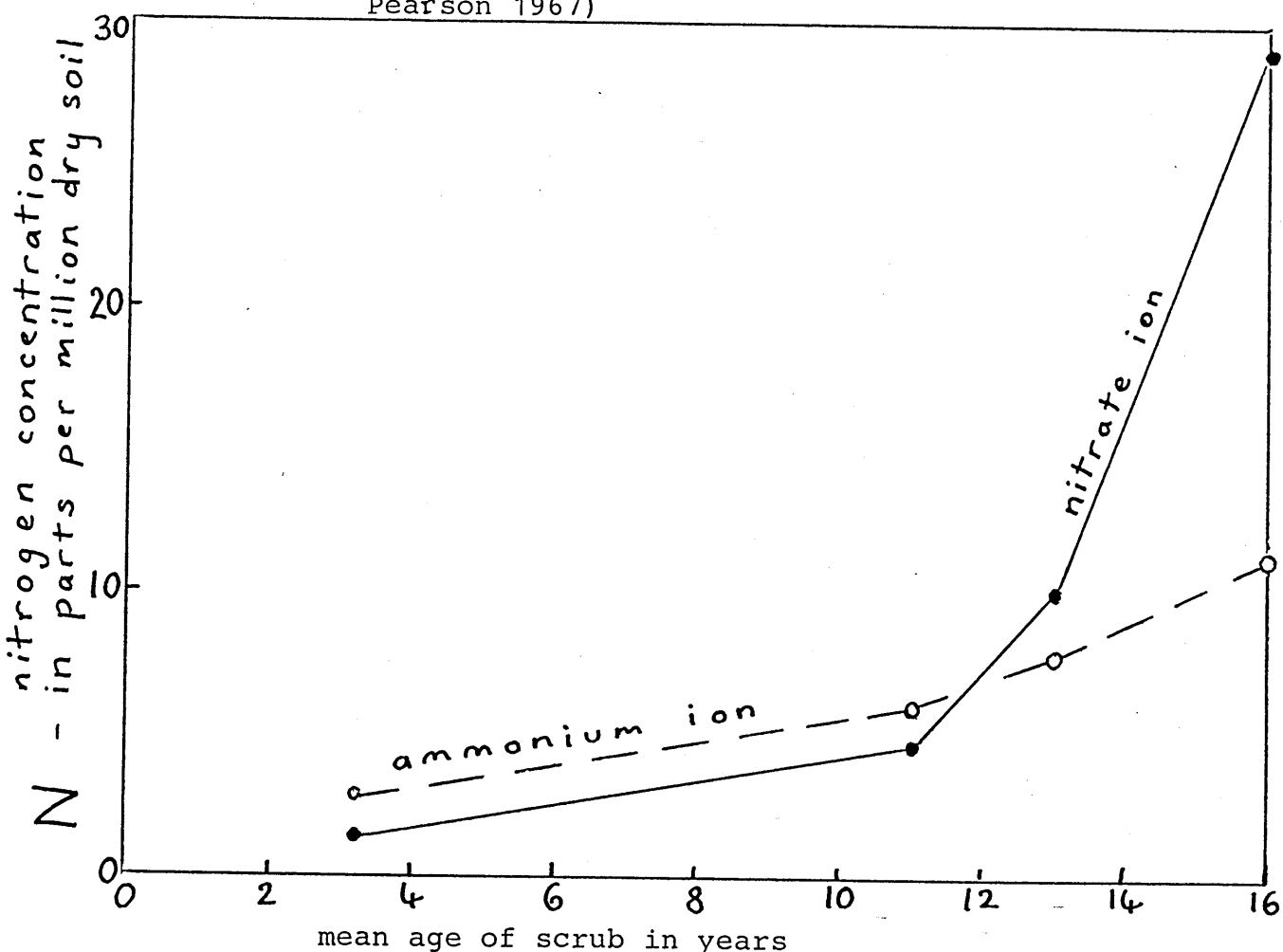


#### 1.6.9 Soluble nitrogen in soil

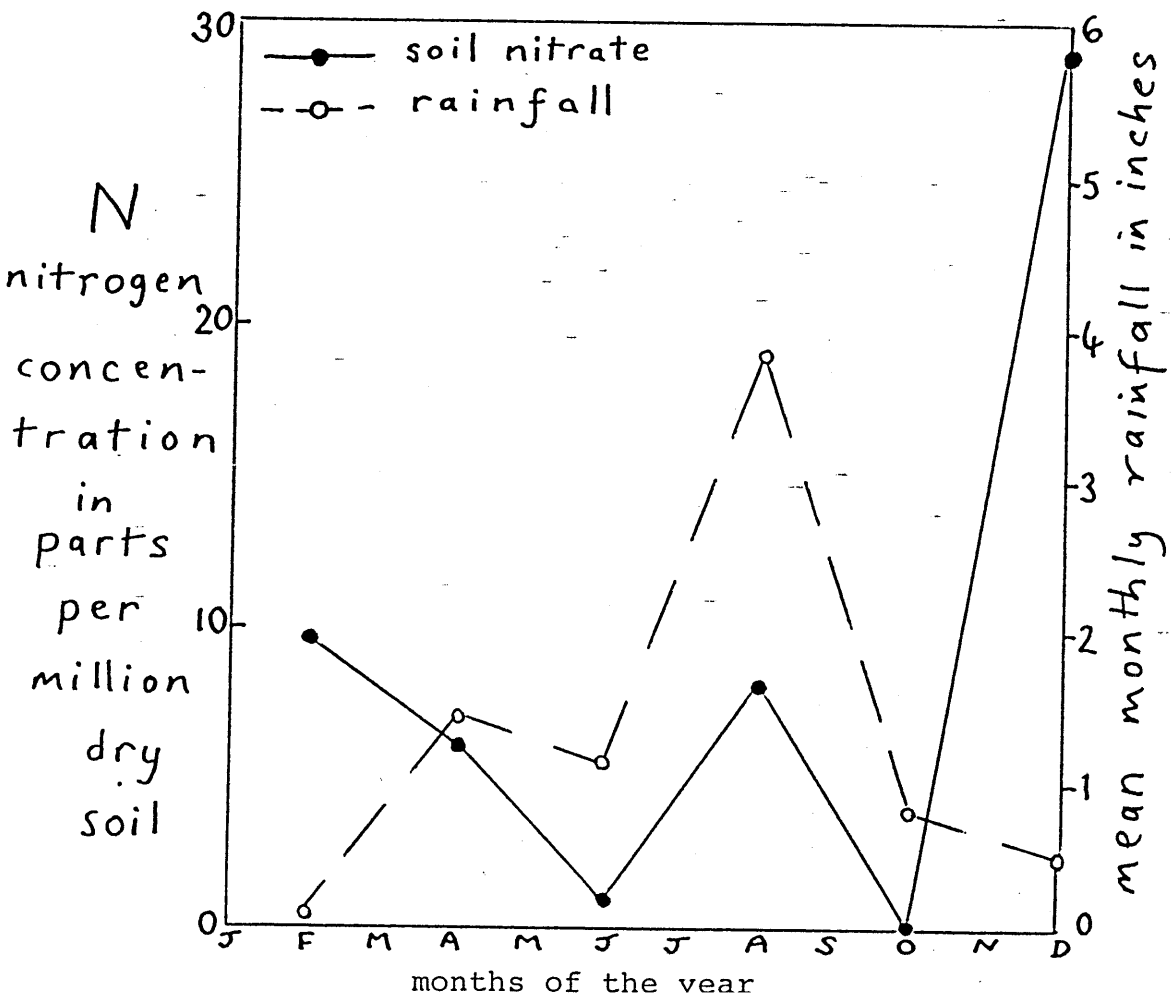
Finally the authors measured the levels of nitrogen-bearing ions within the soil of the four sampling sites, and were able to demonstrate a seasonal variation in ammonium and nitrate ions. These results were presented in Table 6 (p. 355) and are displayed in Graphs 1.34 to 1.37.

The results "show a marked seasonal variation, with maximum values being obtained in the winter months and minimum values in the summer" (p. 355). Although this is broadly true there are curious discrepancies. Values are generally high in February and fall towards June. At Site I nitrate rises from a low February value. At Site II nitrate also rises to an April peak and then declines. Broadly, there is a December peak followed by a possible April peak, and a small August peak. The authors explained the results in terms of the general observations quoted earlier and also by saying "both forms of inorganic nitrogen increase up the dune system" (p. 355) that is, the levels of soil nitrogen increase with the age of the dune scrub. This is demonstrated in Graph 1.38 which shows a plot of ammonium and nitrate concentrations at Sites I to IV in December, because the values are highest at this time of year and likely to be most accurate. The nitrate seems to increase exponentially while the ammonium only increases linearly. Possibly this is due to the ease with which ammonium ion is converted to volatile ammonia.

Stewart and Pearson drew attention to the effects of rain. "In general there is an inverse correlation between the seasonal variation in rainfall and inorganic nitrogen



Graph 1.39 Comparison between annual variation in soil nitrate and rainfall



levels." (p. 355) The extent of this correlation is shown in Graph 1.39 and the authors argued that "assimilation of inorganic nitrogen by the plants undoubtedly also contributes to the low levels during the summer" (p. 356).

Reference was made to other nitrogen-fixing organisms such as *Ammophila arenaria* which were also capable of contributing to levels of dune soil nitrogen. This made it difficult to determine with certainty the exact amounts of nitrogen fixed by *Hippophaë*. Some nitrogen was also lost from the soil by leaching, and de-nitrification also produced losses of unknown magnitude. "Despite these limitations there is no doubt that large increases in total nitrogen do occur in the presence of *Hippophaë* bushes and that these are comparable with gains obtained in arable regions in the presence of a good leguminous crop" (p. 357).

The authors went on to conclude that "it appears likely therefore that fixation increases to a maximum in bushes more than 13 but less than 16 years old" (pp. 357-8). This seems doubtful, given our present knowledge about the relatively short life of *Hippophaë* root nodules before they disintegrate.

However this important series of wide-ranging experiments on *Hippophaë* point to the need for a clearer understanding of what happens to the root nodules within the soil. Much of this clarification emerged from a series of studies by Oremus, which end with some criticisms of the methods and conclusions reached by Stewart and Pearson. These are discussed in succeeding sections.

- 1.7 Variation in the development and decay of the root nodules of Hippophaë
  - 1.7.1 Developmental stages of the endophyte of Hippophaë
  - 1.7.2 Nodulation of Hippophaë under field conditions
  - 1.7.3 Area distribution of nodules
  - 1.7.4 Variation in nodules with age
  - 1.7.5 Necrosis in nodule tissue
  - 1.7.6 Soil composition and root nodules
  - 1.7.7 Discussion of results

## 1.7 Variation in the development and decay of the root nodules of Hippophaë

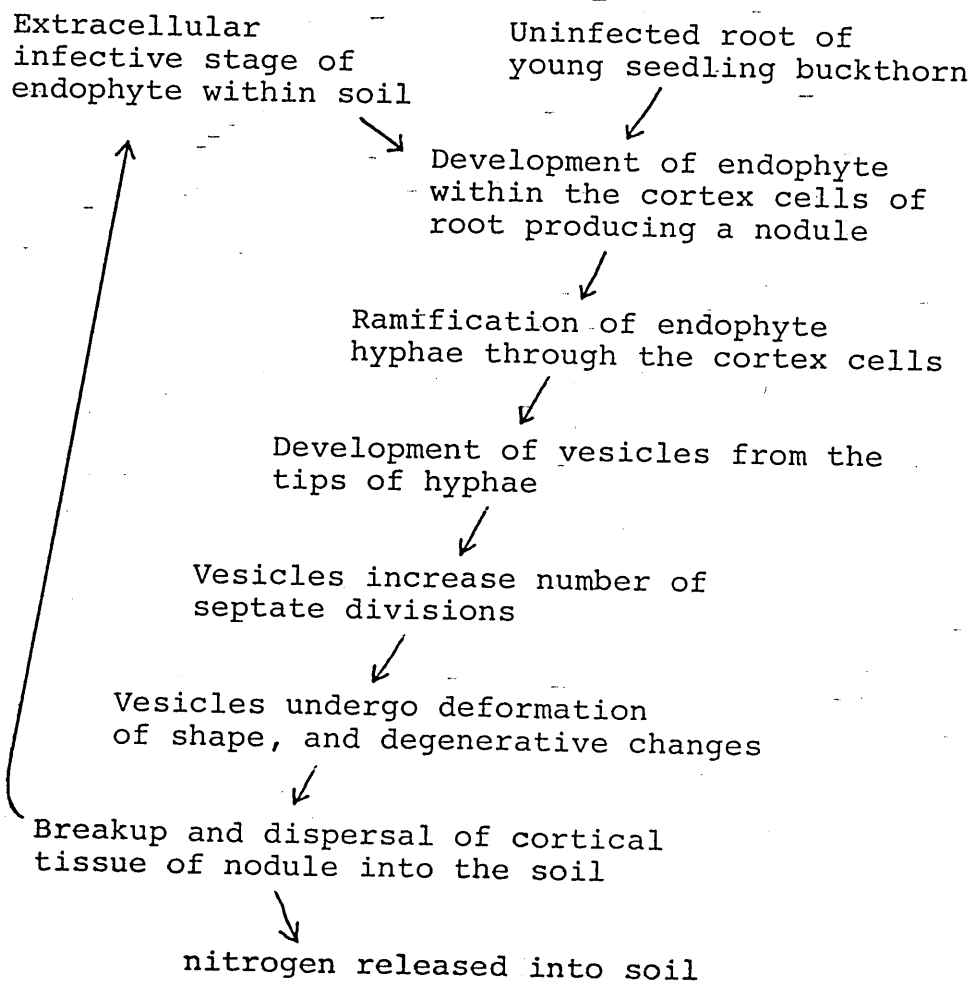
As much of this section is concerned with the breakdown of root nodules under field conditions, a brief account of the histology and cytology of the process is included here. The figures have been drawn by the author from published electron photomicrographs from British and Russian sources.

### 1.7.1 Developmental stages of the endophyte of Hippophaë

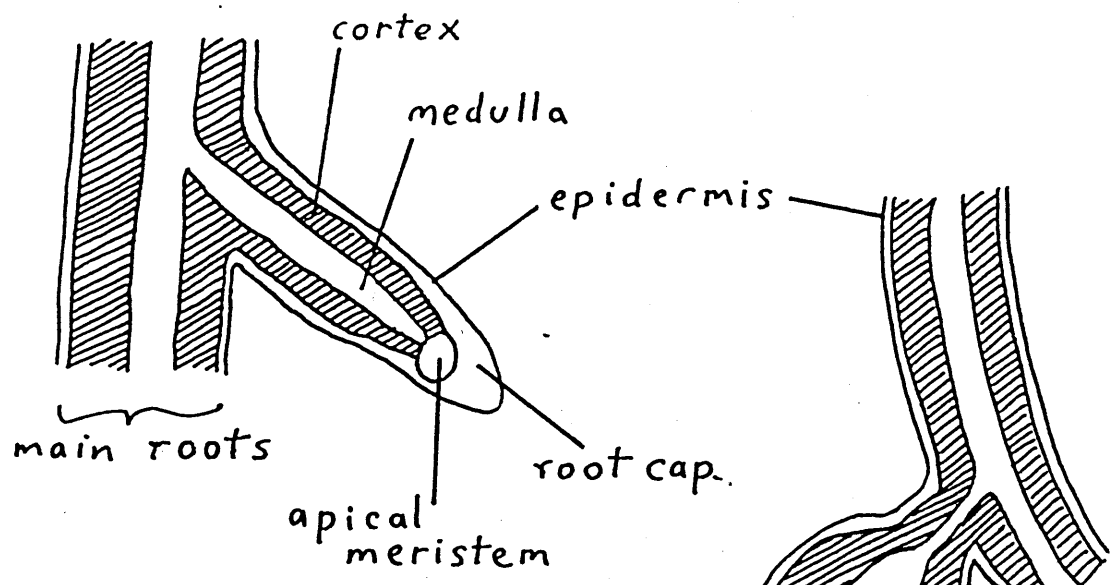
The general pattern of nodule development is indicated below

Figure 1.3

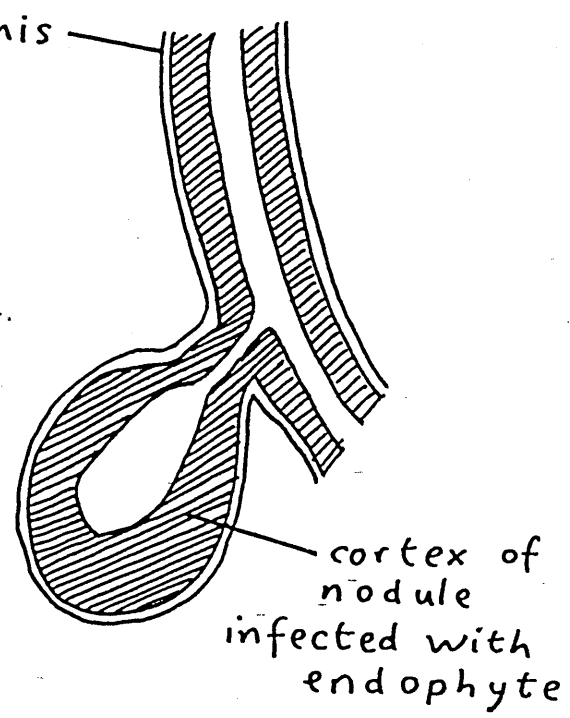
#### STAGES IN THE DEVELOPMENT OF SEA BUCKTHORN NODULES



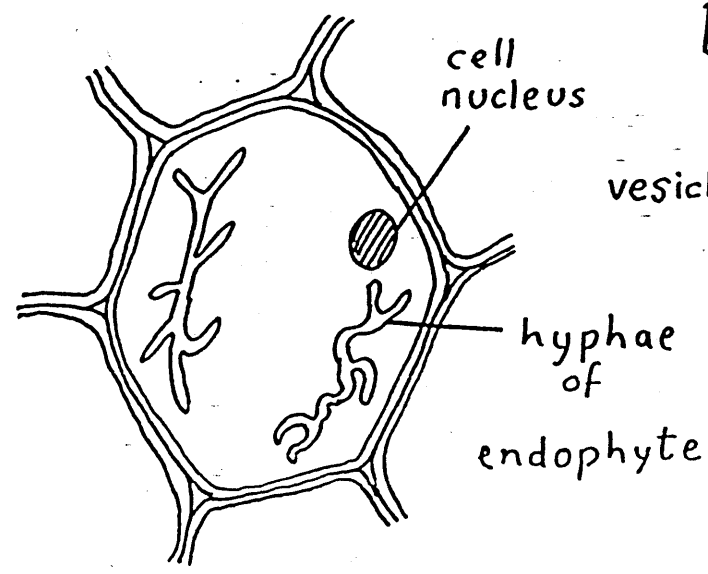
# FIG. 1.4 DEVELOPMENTAL STAGES OF THE ENDOPHYTE OF HIPPOPHAË



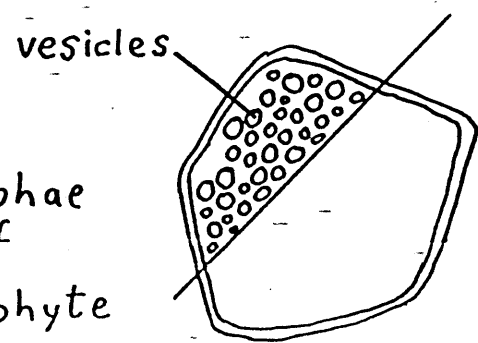
A. L.S. LATERAL ROOT  
x 8



B. L.S. ROOT NODULE x 20

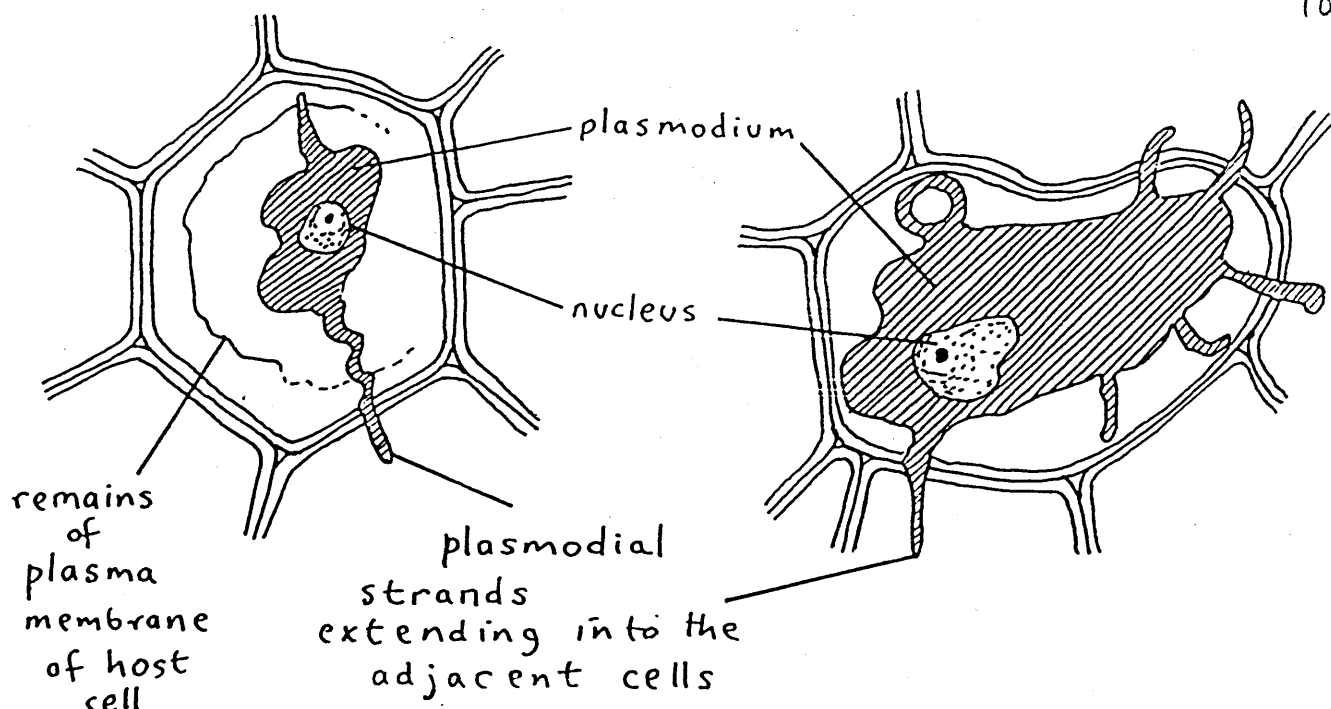


C. CORTICAL CELL EARLY STAGE OF INFECTION  
x 800

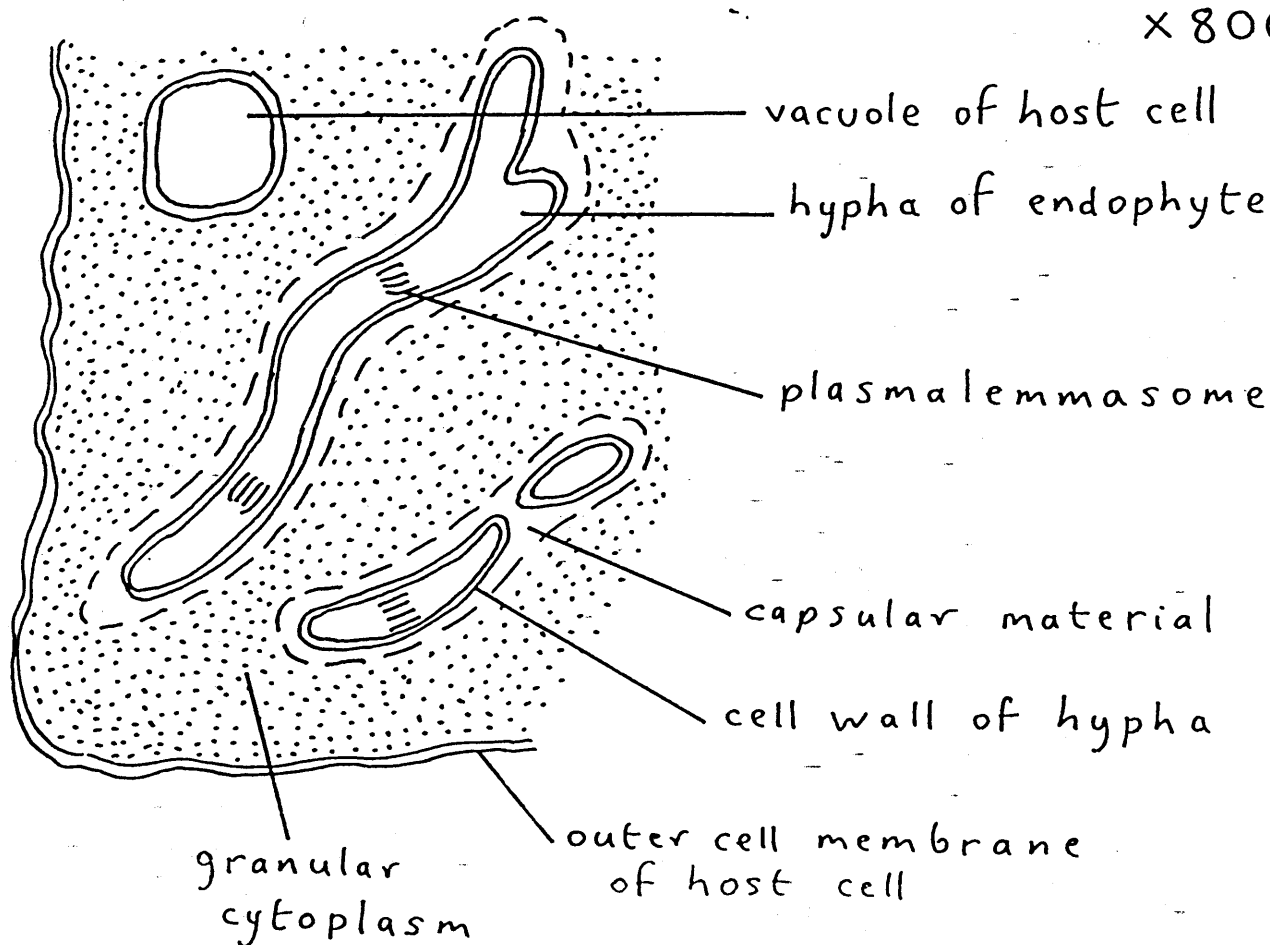


D. CORTICAL CELL AT VESICULAR STAGE  
x 800

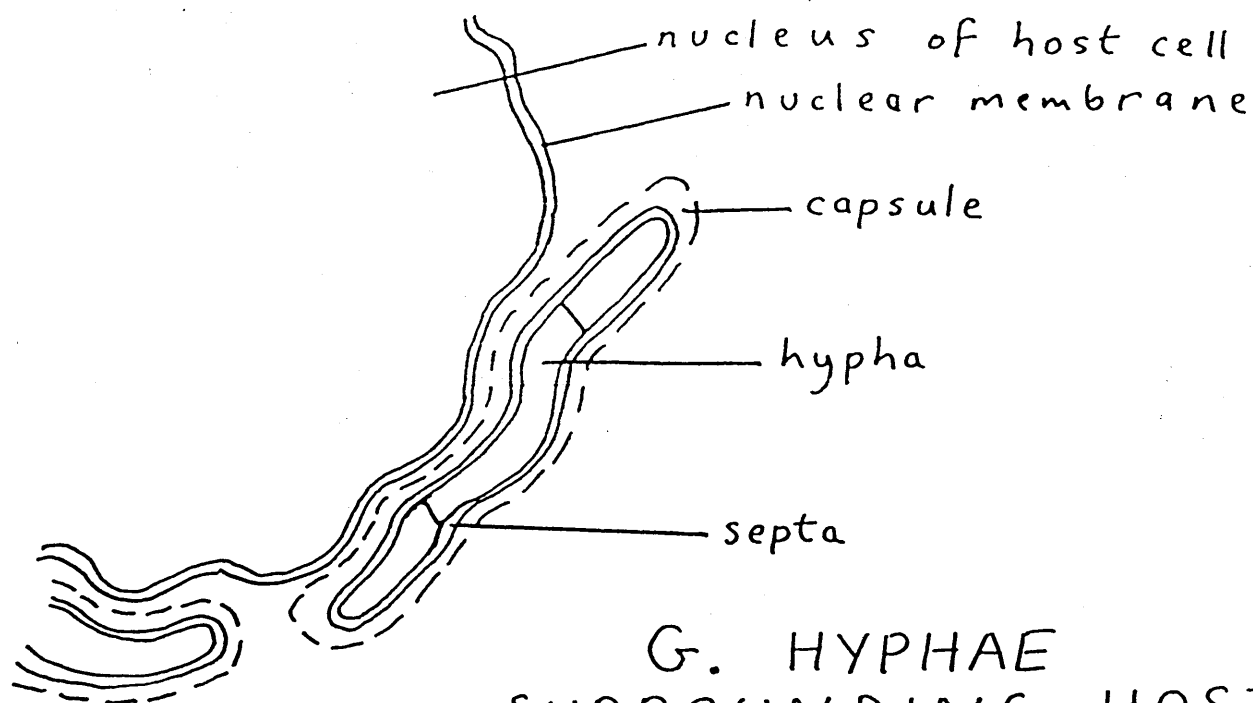




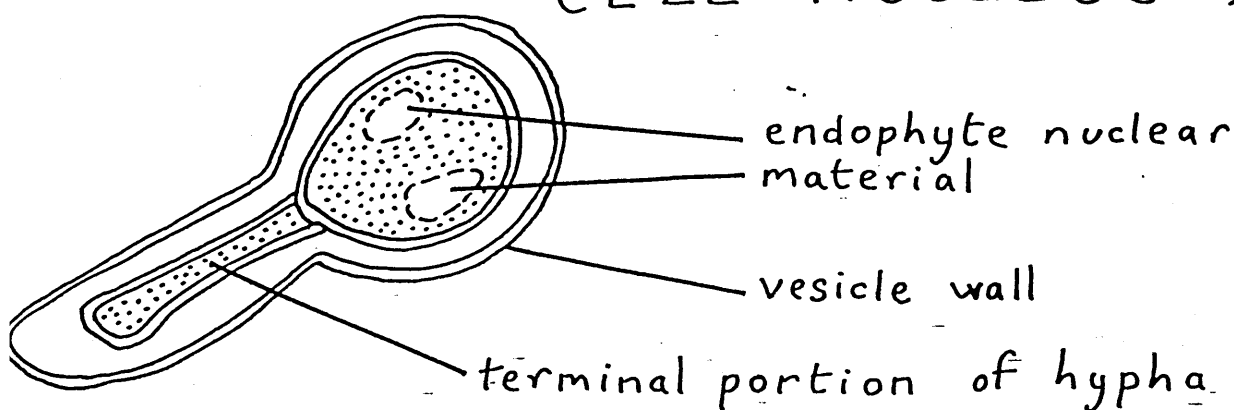
### E. EXTENSION OF ENDOPHYTE WITHIN APEX OF NODULE X 800



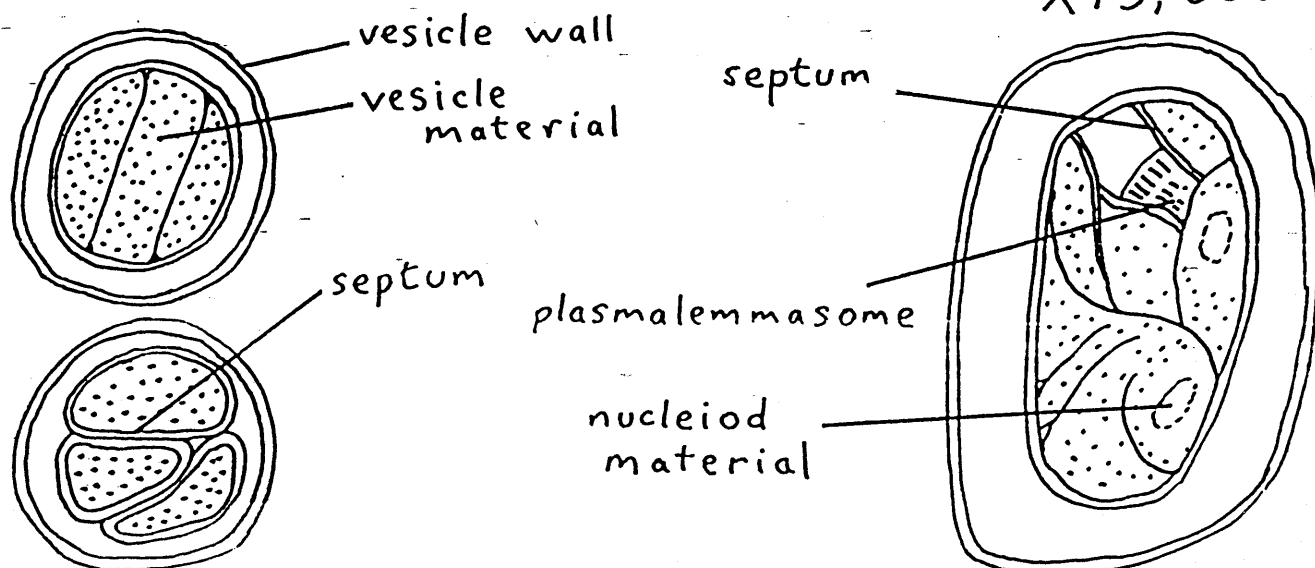
### F. HYPHAE OF ENDOPHYTE WITHIN HOST CELL X 20,000



G. HYPHAE  
SURROUNDING HOST  
CELL NUCLEUS X 8,000

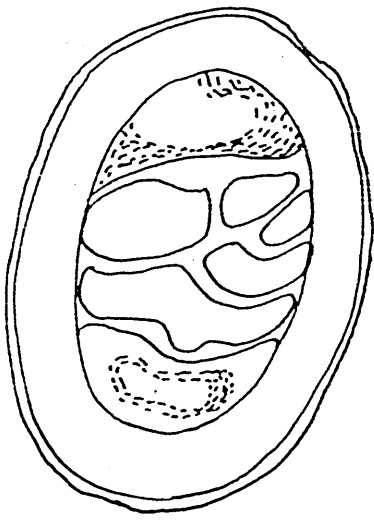


H. DEVELOPMENT OF VESICLES  
FROM ENDOPHYTE HYPHAE  
X 15,000

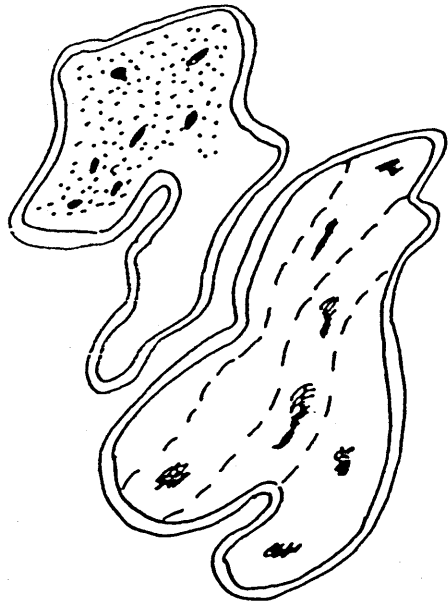


I. YOUNG  
VESICLES

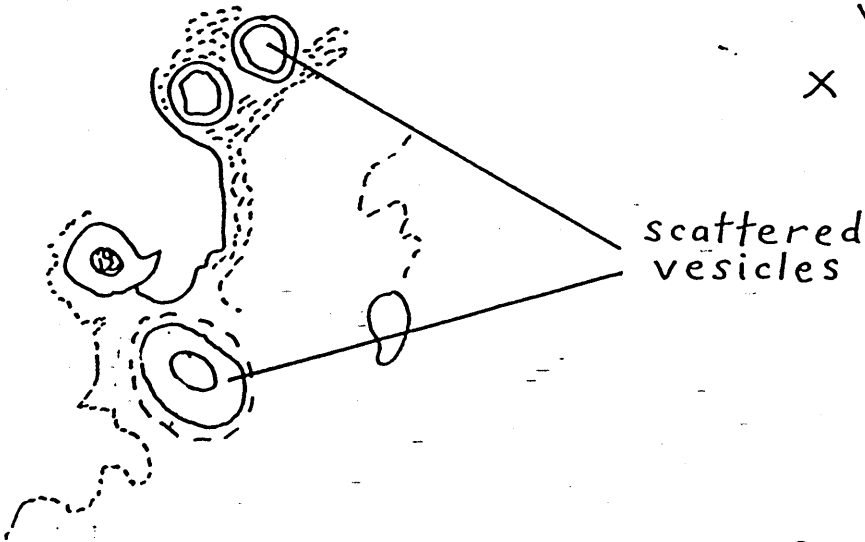
J. MATURE  
VESICLE X  
30,000



K. POST MATURE  
VESICLE  
X 25,000



L. DEFORMED  
AND  
DEGENERATING  
VESICLES  
X 15,000



M. WHOLLY NECROTIC  
TISSUE OF  
NODULES X 10,000

C-J from GATNER AND GARDNER 1970

K-M from ANDREEVA 1979  
ET AL

all figures redrawn from published electron  
photomicrographs

### 1.7.2 Nodulation of *Hippophaë* under field conditions

The process of nodulation is central to nitrogen-fixation under field conditions. This is known to be a very variable and uncertain matter. Hence the value of a detailed quantitative study of nodulation in *Hippophaë* growing in coastal dunes in the Netherlands (OREMUS 1979). The author commented that "despite the ecological significance of *Hippophaë rhamnoides* in ... temperate areas, nodulation in the field has only been described in detail in two reports." (p. 60).

Oremus was referring to a thesis by Akkermans (1971) and a paper by Stewart and Pearson (1967). The authors of the latter drew attention to "the very large discrepancy in the results obtained" (at one particular site) and discussed the need for a "closer scrutiny" of these field conditions. (STEWART and PEARSON 1967 p. 357).

Oremus made a detailed study of sea-buckthorn within a square 3 x 3 metres in coastal dunes near the Hague, Netherlands. The ground was dug up to a depth of 1.5 m so that roots and nodules could be accurately mapped. Shoots were age-dated by cutting sections and counting annual rings. A random sample of 20% of root nodules was age-dated (from the annual rings on their attached roots). Soil samples were taken at depths of 15-40 cm and analysed for pH,  $K^+$ ,  $Na^+$ ,  $Cl^-$ , P,  $CO_3^{-2}$ , total N (Kjeldahl) and organic matter (loss on ignition).

### 1.7.3 Area distribution of nodules

The large (3 m by 3 m) square was sub-divided into 36 smaller squares, each  $0.25 \text{ m}^2$ , and each of these was assayed individually for the distribution of root-nodules, at successive sections of 20 cm depth down to 1.2 m.

The results for each square were shown diagrammatically in a multiple bar chart (OREMUS Fig 2 p. 63). The advantage of this method of display is that the extreme variability is immediately apparent. The author did not give the figures because most of the data were presented later in grouped frequency tables. He did not comment directly on his Fig 2 so it is perhaps worth noting the main things which emerge.

- (i) Some squares have no nodules, and others have very few, but some have very many.
- (ii) In some squares the nodules are concentrated at the surface, and in a few they occur at depth.


Oremus displayed the information from his Fig 1 as a grouped frequency table in his Table 1. He grouped all the depth variations for each square, and cited a total nodule number for each of the 36 squares. These data are presented in my Table 1.17. A simplified contour diagram helps to make the point that the nodule distribution is largely random; (Fig 1.5) as the author comments "the number of nodules in this homogeneous scrub differed widely from place to place " (p. 61).

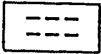
Table 1.17 Plan of root nodule number in a 3 metre by 3 metre stand of *Hippophaë* (data from Oremus 1979)

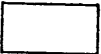
106	63	32	4	9	0
60	52	28	115	21	0
6	39	19	36	23	13
2	16	9	32	18	19
0	35	31	35	28	0
0	2	14	4	12	0

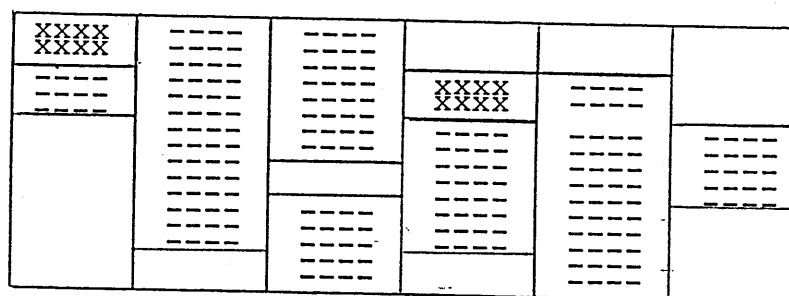
Figure 1.5 Plan of root nodule number

KEY

 > 100

 11 to 99

 < 10

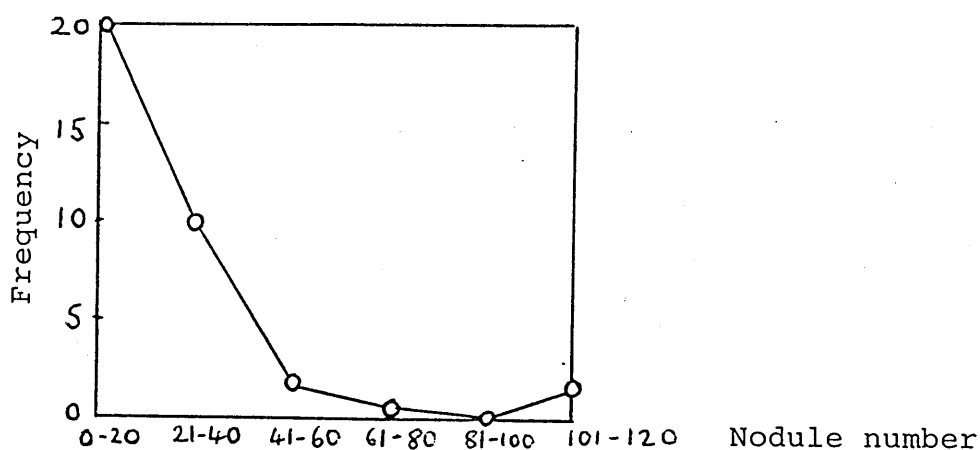


The relationship between nodule-number and frequency is shown in Table 1.18 and Graph 1.40. Clearly there are far more sample sites with low nodule-numbers than those with high numbers. This bears out the case presented by Oremus, that without a large number of samples one may easily underestimate the extent of nodulation, or at least, misrepresent it (p. 65).

Table 1.18 Frequency of nodule-numbers in quarter metre squares. (data from Oremus 1979)

Nodule-number	0-20	21-40	41-60	61-80	81-100	101-120
Frequency	20	11	2	1	0	2

Graph 1.40 Frequency distribution of nodule-number



#### 1.7.4 Distribution of nodules with depth

Oremus re-presented the comprehensive results from his Fig 2 in an alternative grouped frequency table, this time to show the variation in distribution of root nodules with depth of soil. He combined the data from all 36 squares for each of the seven depth layers, and computed the means, the ranges and the total nodule number as a percentage of the whole plot. His data are shown in Table 1.19 slightly re-arranged. The close agreement between the percentage nodule number, and the absolute nodule number per  $m^2$  is clearly evident in Graph 1.41.

The maximum number of nodules occurs at a layer between 20 and 40 cms depth, and 83% of all nodules are found between 10 and 50 cm. These findings have an important bearing on the appropriate depths at which to sample for nodules or soil. The limitation of nodules at the surface is probably because "the upper layer of 10 cm is drier than the lower layers" (OREMUS p. 61).

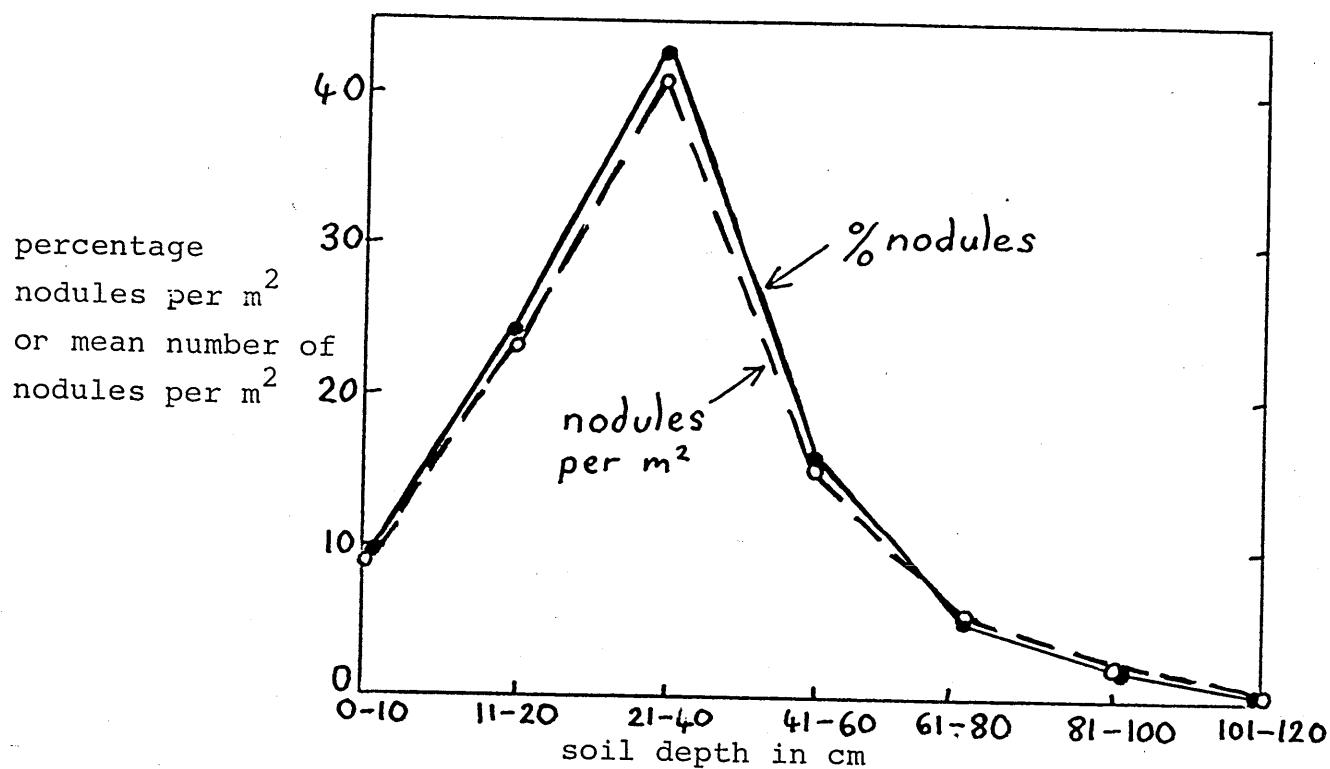
Table 1.19 Distribution of root nodules of Hippophaë with soil depth. (data from Oremus 1979)

Depth cm	% nodules	Nodules per m <sup>2</sup>	
		mean	Range
0-10	8.7	8.7	0-34
11-20	24.2	23.4	1-60
21-40	42.6	41.2	17-125
41-60	15.9	15.4	0-48
61-80	5.7	5.5	0-21
81-100	2.3	2.2	0-17
101-120	0.6	0.4	0-4

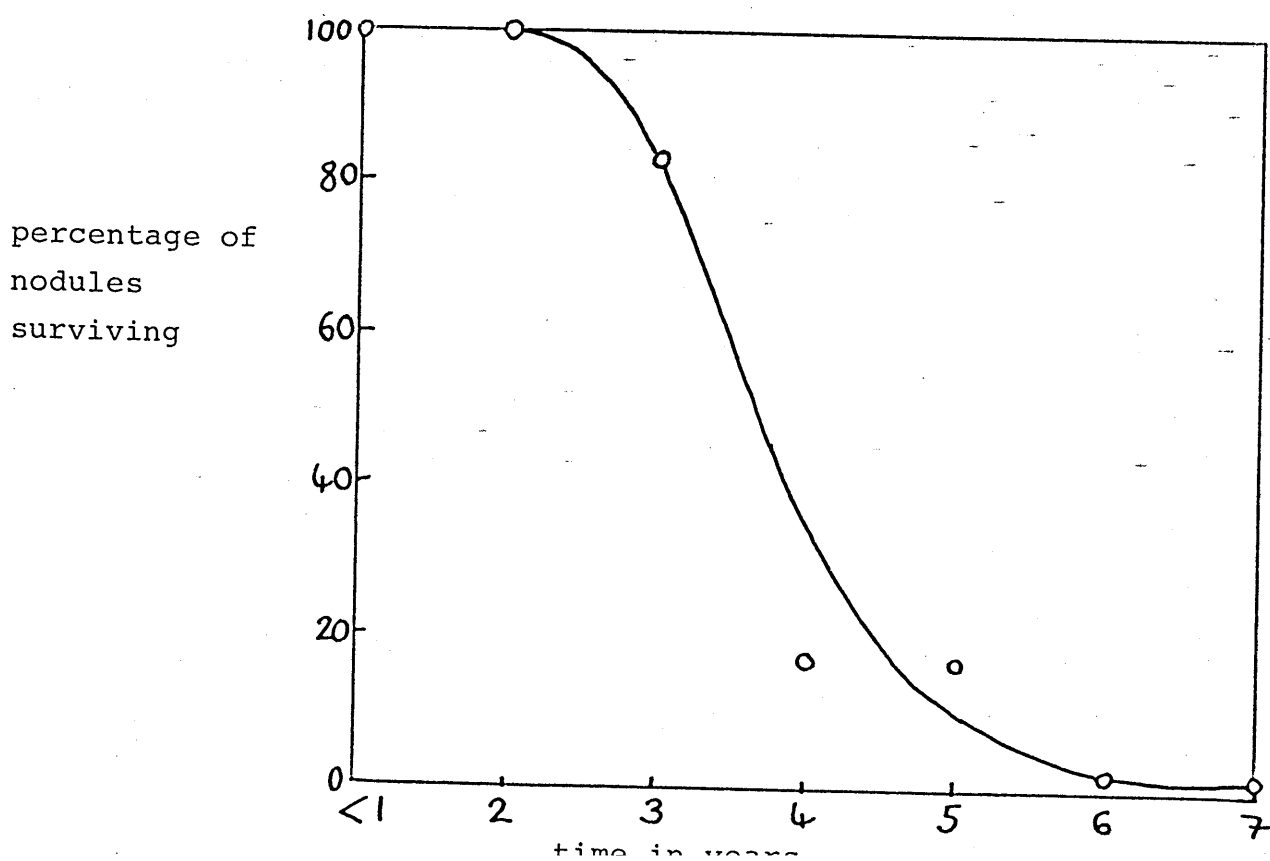
KEY: % nodules = percentage of the nodules from the whole plot, present in that layer



Graph 1.41 Variation in nodule frequency with depth of soil (data from Oremus 1979)



Graph 1.42 Putative survivorship curve for root nodules of Hippophaë. (data from Oremus 1979)



#### 1.7.4 Variation in nodules with age

Oremus was interested in obtaining information on "the course of nodulation of these plants" and his results, presented in his Table 3, "show a sharp decrease in the number of nodules in the age classes of 4 years and older, which indicated that many of them die after 3 years" (p.63).

He went on to contrast the living and dead nodule material; "these young nodules are the most active in di-nitrogen reduction and the efficiency decreases with age. The decrease in the amount of living nodule tissue after 3 years, is due to the death of whole nodules, as well as parts of nodules, which is not compensated for by newly formed material". (pp. 63-4).

The data from his Table 3 are given in my Table 1.20, with the addition of two extra columns (8). In this short paper he limited himself to a discussion of the results, but I have taken the opportunity to present some of his data in graphical form.

It has to be remembered that the experiment was dealing with frequency data, because there is a temptation to see the results as a time-course.

In Table 4 - N% it can be seen that about  $\frac{1}{3}$  of the nodules were a year old,  $\frac{1}{3}$  were 2 years old, and just under  $\frac{1}{3}$  were 3 years old. Less than  $\frac{1}{10}$  were over 3 years old. This is surprising because one would expect there to be more of the youngest nodules, and the numbers to decline from there downwards. If these proportions were steady from year to year, and this assumption is doubtful, then about  $\frac{1}{3}$  of the nodules

would die each year, and a similar number would develop in replacement.

Table 1.20 Age-variation in nodules of Hippophaë (data from Oremus 1979)

1  age years	2	3	4	5	6		7	8	
	NUMBER OF NODULES		TOTAL NODULE WEIGHT		WEIGHT PER NODULE		NECR- OTIC NODULE TISSUE	LIVE NODULE TISSUE	
	Na	N%	Wa	W%	$W_n$		$W_d\%$	$W_l$	$W_l\%$
1	60	31.4	1810	16.8	$30.2 \pm 10.5$		2.2	1770	18.7
2	62	32.5	5263	48.9	84.9	32.1	2.3	5142	54.3
3	50	26.2	2497	23.3	49.9	23.3	13.5	2160	22.8
4	8	4.2	498	4.6	62.3	65.7	70.8	145	1.5
5	9	4.7	656	6.1	72.9	38.7	63.2	241	2.6
6	1	0.5	35	0.3	35.0		92.1	3	0.0
7	1	0.5	3	$\leq 0.1$	3.0		80.0	0.6	0.0
TOTAL	191	100.0	10,762	100.0	-		-	9462	99.9

#### KEY TO COLUMNS

- 2 Na - absolute number of nodules in each age class
- 3 N% - nodules in each age class as a percentage of the total
- 4 Wa - absolute weight of nodules in each age class of mg of dry weight
- 5 W% - weight of nodules as a percentage of total
- 6  $W_n$  - weight per nodule - (living tissue) mean + 1 standard error of deviation
- $$W_n = \frac{Wa}{Na}$$
- 7  $W_d\%$  - necrotic nodule tissue - as a percentage of the sample in each age class
- 8  $W_l$  - weight of live nodule tissue mg of dry wt.

$$W_1 = \frac{100 - W_d\%}{100} \times W_a$$

9  $W_1\%$  - weight of live nodule tissue as a percentage of the total

$$W_1\% = \frac{W_e}{\sum W_e} \times 100$$

The frequency distributions of the age-groups of nodules may correspond to a time-course for a few years. Hence it is possible to represent this by converting the nodule frequencies  $N_a$  to a survival curve, that is number of nodules surviving as a percentage of the original number.

( $N_s\%$ ) This is given below in Table 1.21

Table 1.21 Putative survivorship curve for root-nodules of *Hippophaë* (data derived from Oremus 1979)

Age years	1	2	3	4	5	6	7
$N_a$	60	62	50	8	9	1	1
$N_a$ approx	60	60	50	10	10	1	1
$N_s\%$	100	100	83	17	17	2	2

The data have been roughly approximated to avoid the problem of survivorship increasing from one year to another. The curve is shown in Graph 1.42\* indicating a sharp decline in survivorship between the third and fourth year. The value of presenting the data in this form is that it facilitates comparisons between different studies. The only way to validate a time-course like Graph 1.42 is to carry out frequency sampling each year, to see if the proportions remain constant. Given our knowledge of the maturing of

sea-buckthorn this seems unlikely.

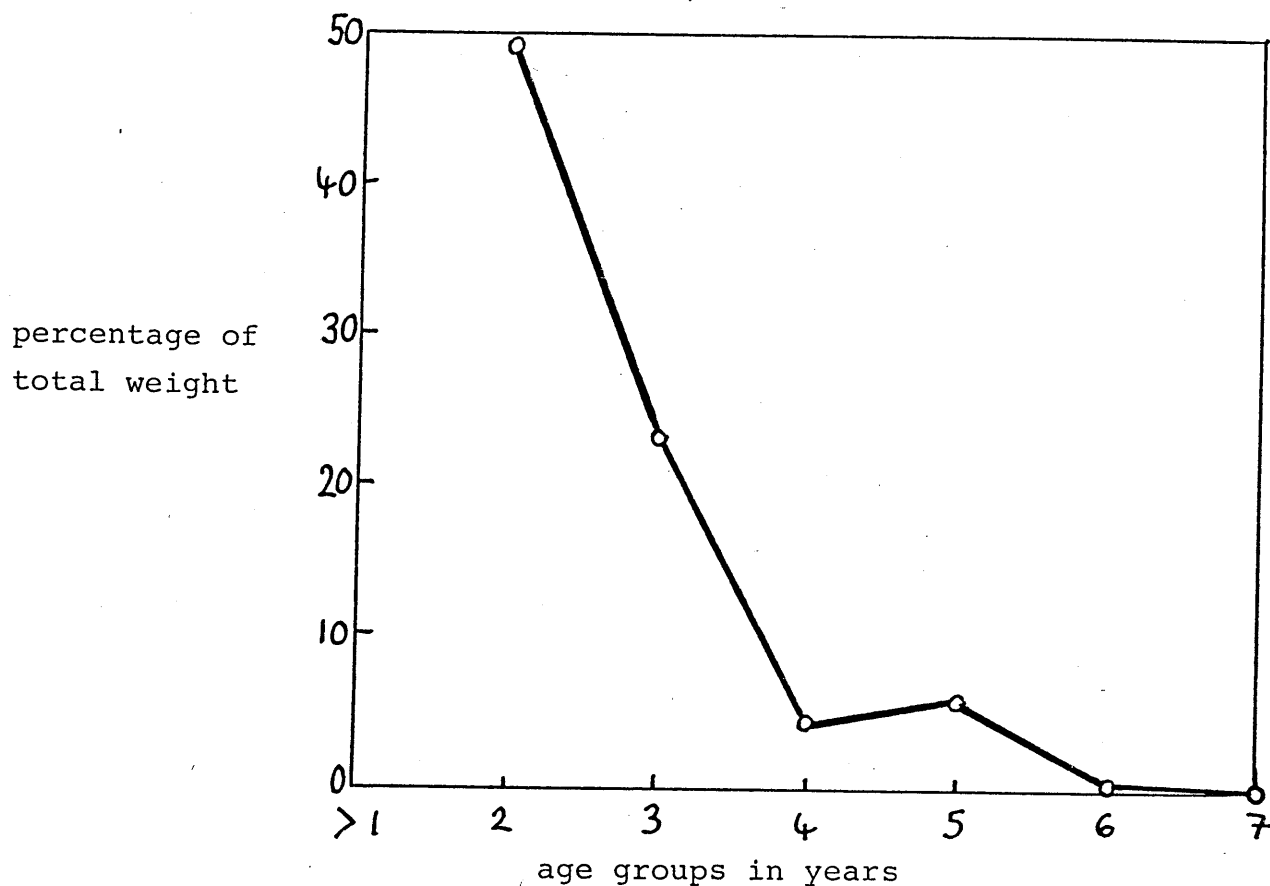
The data for total nodule weight  $W_a$  is possibly a better parameter to study because it reveals more about the amount of nitrogen-fixing material present, than a simple count of nodules. If the nodules change in size with age then nodule number is only a rough approximation of the nitrogen-fixing capacity. Given that the number of nodules in the 1-year and 2-year age groups was almost the same (60 and 62 respectively) the difference in weight of nodule material  $W_a$  indicates that the 2-year nodules were  $2\frac{1}{2}$  times the size of the one-year nodules.

The figures in Table 1.19 show that half the weight of nodular material is two years old, and 90% of it is 3 years old or less. This is a most striking demonstration of the short life of root-nodules.

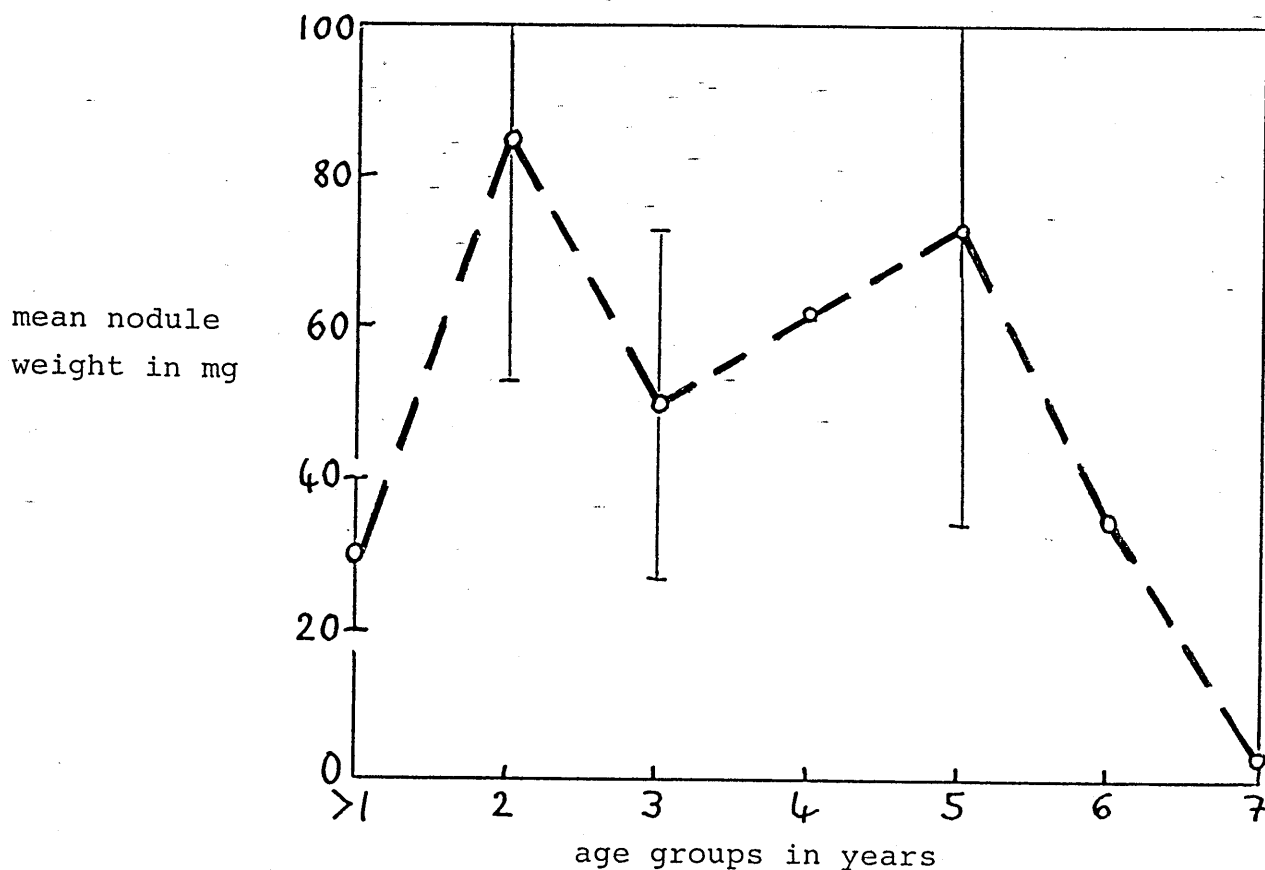
The frequency distributions of the age classes are shown in Graph 1.43. It is possible that a time-course for the nodule-weight of a particular year class would follow the same basic pattern. That is, nodulation would develop in the first year of root growth, when infection by the endophyte occurred at a group of points along the root. During this year the nodules would increase in size. By the end of the year, and into the next, the number of nodules would remain constant, but they would increase in size by two or three times. Into the third year, the nodules would decline in size and some would die off. By the fourth year most of the nodules would be dead, and they would continue to decrease in size.

This short account is confirmed by the data on  $N_a$ ,  $W_a$  and  $W\%$  in Table 1.20. However the findings on average nodule weight

Graph 1.43 Distribution of root nodules among age groups as a percentage of the total nodule weight (data from Oremus 1979)



Graph 1.44 Distribution of mean nodule weight among age groups (data from Oremus 1979)



are not so clear. Oremus could have calculated this by the formula  $W_n = \frac{W_a}{N_a}$  but it seems that he used a different method, because he quotes a mean and standard error of deviation. Possibly the figure is based on a mean of all 36 small sample squares.

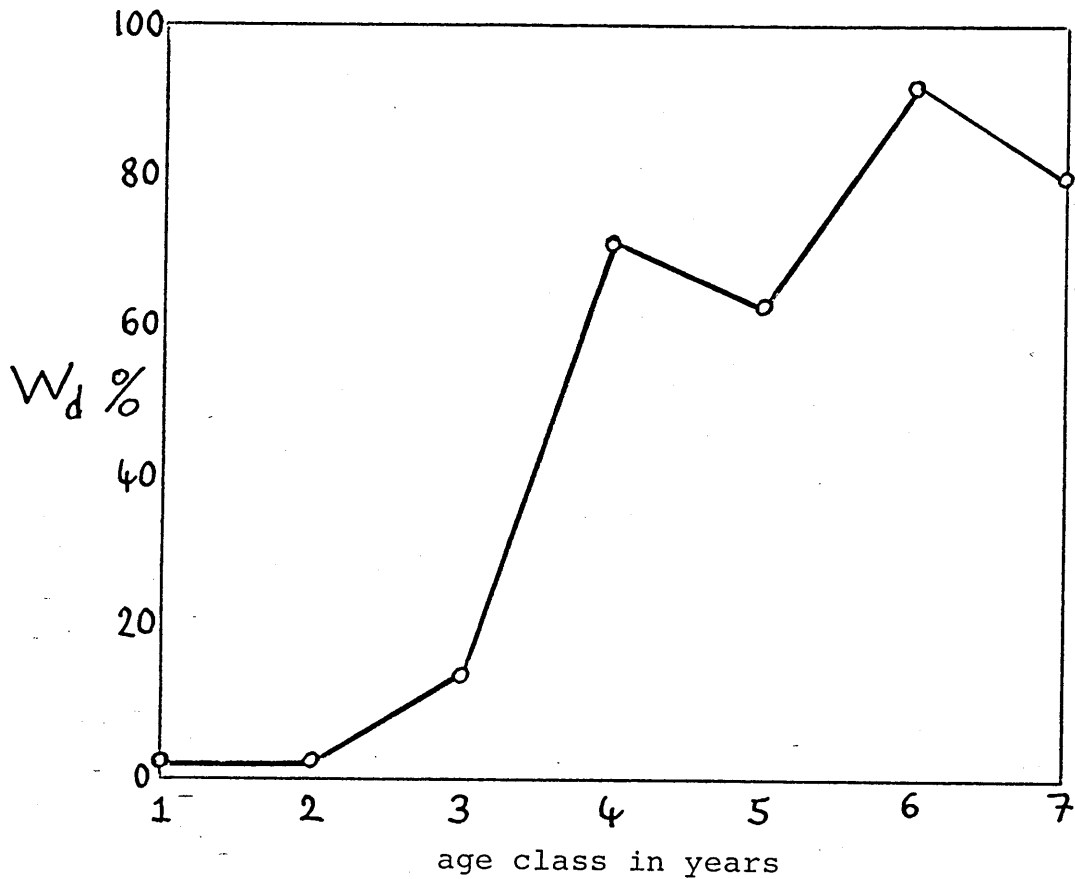
The values of  $W_n$  seem to fluctuate rather irregularly (Graph 1.44) but given the size of the standard error it is not possible to put much confidence in any trends which may be revealed. Oremus considered that no relationship could be found "because of the great variation between nodule weights in each age class" (p. 64).

#### 1.7.5 Necrosis in nodule tissue

Oremus had realised that the death of nodular tissue was going to affect the overall level of nitrogen-fixing capacity, so he attempted to estimate the degree of necrosis, and his results are given in column 7 of Table 1.20. The general trend is shown in Graph 1.45 where necrosis is seen to be minimal in the two youngest year-classes, around 2%, increasing in the 3 year group, and remaining high at 70-90% in all the older nodules.

A series of annual samplings of sea-buckthorn nodules would be necessary to show if this frequency distribution in Graph 1.45 corresponded to a time-course for nodule necrosis. The rather variable values for necrosis after year 3 is probably explained by the very low nodule numbers present. The general picture is consistent with earlier findings; not only does the nodule number, and nodule weight decline after 3 years, but a much bigger proportion of the nodules show necrosis.

Graph 1.45 Necrosis of root-nodule tissue of Hippophaë  
(data from Oremus 1979)



Key:  $W_d$  % = necrotic nodule tissue as a percentage of the sample in each age class



As nitrogen-fixation depends upon the live tissue it seemed worthwhile to extract the information on this from Oremus' data.

$$(W_d\% = \% \text{ age of necrotic tissue})$$

$$\% \text{ Live nodule tissue} = 100 - W_d\%$$

$$\text{Actual weight of live nodule tissue} = \frac{100 - W_d\%}{100} \times \text{total nodule weight}$$

$$\text{Frequency of live tissue in each age class as a percentage of the whole } (W_l\%) = \frac{W_l}{\sum W_l} \times 100$$

These values are shown in Table 1.20 and they do not markedly affect the earlier findings, except to emphasize them more strongly.

Live nodule tissue is found in the largest amounts in two-year old roots. The decline of live tissue in nodules over 3 years of age is even more marked than the decline of total nodule tissue.

	Nodule weight as a % of all nodules	Live nodules as a % of live and dead material
Tissue in 2 year-old nodules	48.9%	54.3%
Tissue in nodules of 3 years and under	90%	96%

The percentage frequency distributions of live tissue showed the same trends in a more marked way than those for total nodule tissue.

All the results point to an optimum physiological efficiency for the root-nodules of Hippophaë at two years of age, and

an almost complete decline after four years. This does not tell us how the soil in the vicinity is affected.

#### 1.7.6 Soil composition and root-nodules

Oremus was primarily interested to see if small-scale variations in soil conditions could account for the variability of nodulation. Alternatively, of course, the variations in nodule concentrations could produce different soil conditions, particularly with respect to nitrogen. Whichever way the situation is examined, the first requirement is to show an association between nodules and chemical components of soil. Explanations can follow later. The data are given in Table 1.22 which is a reduced version of Oremus' Table 4 (p. 65) omitting P, K, Na and Cl.

Table 1.22 Chemical components of dune soil in contact with root-nodules of *Hippophaë* (data from Oremus 1979)

Nodulation state	pH	% $\text{CaCO}_3$	% organic	total N*
No nodules	8.2	1.82	0.80	14.0
Few nodules	8.4	2.23	0.66	9.2
Many nodules	8.3	2.33	0.78	20.4

\* in mg per 100 g dry soil = ppm x 10

Oremus gives no details on the replication of his soil samples, or the extent of nodulation. It is not clear whether the data in the table are means or not, or what the standard error might be.

There does not seem to be any significant difference between

the values of three of the parameters, pH, %  $\text{CaCO}_3$  and % organic for the three nodulation states. In the fourth one, total nitrogen the differences are larger, but in the absence of a consistent change, it must be assumed that the variation is purely random, and that no association exists between nodulation and soil chemistry.

#### 1.7.7 Discussion of results

The author compared the concentration of root nodules estimated in his study (30-281 per  $\text{m}^2$ ) with the similar study by Stewart and Pearson in 1967 (98-370 per  $\text{m}^2$ ). Although Stewart and Pearson considered that the differences in nodule numbers were related to the age of the sea buckthorn scrub, Oremus thought that this was a dubious conclusion because "their data are based on observations in plots measuring only 1  $\text{m}^2$ ". He emphasized that the same kind of variation had been observed in his study of "a homogeneous 6-7-year-old stand" (p. 65).

In Akkermans' study of 1-2 year-old scrub nodule numbers varied from 9-200 per  $\text{m}^2$ . In older scrub (4-15 years) the level of nodulation was very low (0-25 per  $\text{m}^2$ ). Oremus again criticised the small samples (1  $\text{m}^2$ ) and restated his opinion that "results obtained in small samples are of little value for the comparison of different stands" (p. 65).

Is this criticism justified? Oremus only examined 9  $\text{m}^2$  himself. The fact that it was one single plot does not automatically confer greater reliability on his results. It is possible that he was observing a localised and atypical area. What is definitely important is the total area

examined. If Akkermans surveyed  $32 \text{ m}^2$  in a single age class of Hippophaë, this provides more statistical reliability than the  $9 \text{ m}^2$  of Oremus. The fact that Akkermans' sample areas were all separated into  $1 \text{ m}^2$  plots does not necessarily affect the reliability. The argument is about random samples as opposed to one single large sample in what is assumed to be a homogeneous region. Without embarking on a fuller examination of the issues it is true to say that the problem is not quite as simple as Oremus suggests.

Further criticisms of the methods of Akkermans and Stewart and Pearson are made concerning the age-dating of nodules. They estimated the age of the shoots by annual ring counts. Oremus points out that in his study the shoots were all 6-7 years old, but the roots varied from 1-7 years. Moreover he found close correlation between root age and nodule number. This is a very valid objection. Dating nodules by shoot age is an indirect process, and Oremus has shown it to be unreliable by the direct method of dating roots to which nodules are attached.

Oremus found the mean nodule weight for the whole survey area to be 6.3 g (5.3 g alive, 1.0 g necrotic). Akkermans found values of 1.4 g to 1.9 g in scrubs of a similar age. Stewart and Pearson found 2.5 g and 39.0 g in scrubs of 3 years and 11 years respectively. Oremus comments that "since in the latter study no distinction was made between necrotic and living nodule parts a significant proportion of the nodule weight of the 11-year-old scrub could have been necrotic" (p. 66). The criticism must surely be correct, given that Oremus found that around 90% of living nodule tissue was less than four years old.

In conclusion Oremus examined three possible explanations of the variations in nodule numbers observed.

(a) The distribution of young roots

He found that young roots arose very randomly from a system of underground stolons. Given that nodules form mostly on young roots their distribution is also going to be random. In addition he noted that in some cases large numbers of young roots produced few nodules, and in other cases, vice versa. There was no obvious correlation between young shoots and nodule numbers.

(b) The existence of local differences in soil conditions

Although his data "do not show significant local differences in either the concentration of some major elements or the pH and organic matter ... it remains possible that still unknown factors inhibit nodule formation on a local scale" (p. 66).

(c) A heterogeneous distribution of the endophyte

The necrotic nodules contain large numbers of infective particles, and since the endophyte of *Hippophaë* has not been observed growing in the soil, it is presumed that infection of young shoots must be from the process of nodule decay. This possibility was the subject of continuing research by the author.

This work has an important bearing on the matter of soil fertility in the vicinity of *Hippophaë*. Oremus has established a "life-cycle" for the root-nodules. What is needed next is an analysis of how release of nitrogen from roots and nodules varies during this life cycle. Do the nodules make a larger contribution to soil fertility before or after their death?

- 1.8 The relationship between the distribution of the endophyte and root nodulation in Hippophaë
  - 1.8.1 Assay for infective potential
  - 1.8.2 Changes in I.P. with storage
  - 1.8.3 Variation in infective potential of dune soils
  - 1.8.4 Infective potential at successional extremes
  - 1.8.5 Inhibition of nodulation

### 1.8 The relationship between the distribution of the endophyte, and root nodulation in Hippophaë

One of the important problems to emerge from the 1979 paper by Oremus was how to account for the great variation in the spatial distribution of root nodules in Hippophaë. He had suggested that the explanation might lie in either randomly distributed chemical factors or in the heterogeneous distribution of the endophyte associated with nodule development. His subsequent research was designed specifically to examine these two hypotheses. (OREMUS 1980)

The principal hypothesis was "that the input of infective endophyte particles into the soil is largely determined by their release from decayed nodules." (p. 123-4) The author coined the term infective potential (I.P.) to describe the "minimum number of infective endophyte particles in the soil" (p. 124). He assayed I.P. by growing Hippophaë seedlings in contact with test material for a period and then counting the number of nodules which formed. The first series of experiments were designed to validate the assay procedure.

#### 1.8.1 Assay for infective potential

Nodule homogenates were prepared by blending known dry weights of root nodules in known volumes of nitrogen-free aqueous media. Hippophaë seeds were collected, surface sterilized, germinated, and then grown for five weeks in sterile culture, in readiness for infection.

A series of dilutions of the original nodule homogenates were prepared, and then used as culture media for the

uninfected *Hippophaë* seedlings. The culturing continued for five weeks only, as preliminary studies had indicated that no new nodules form after a five-week period. The number of nodules formed on the seedlings was counted, and the infective potential was estimated on the assumption that one nodule is equal to at least one infective particle of endophyte.

Oremus had written a short paper, and he confined himself to publishing the results in Table 1 (p. 127) and presenting the main conclusions. His estimates of I.P. in the nodule samples ranged between  $1.0 \times 10^6$  and  $4.7 \times 10^6$  particles per gram of air-dried powder "which indicates that the method is sufficiently reliable to be used in a quantitative study of the I.P. of *H. rhamnoides* nodules" (p. 126).

The results of his Table 1 are given a fuller graphical analysis here. Each of the six experiments yielded a different value for I.P. based on the mean of four replicates at six different concentrations. The results have been grouped to show the I.P. at each concentration based on the mean of four replicates each of six experiments (Table 1.23). Graph 1.46 shows clearly that the dilution of the original homogenate produces a proportional reduction in the infective potential. Such regularity in the log x log plot of nodule weight W against Log I.P. suggests that the assay method devised by Oremus is very reliable, given that a suitable number of replicates are employed.



Table 1.23 Variation in infective potential with different concentrations of nodule homogenate (data from Oremus 1980)

W	$N_t$	$N_m$	I.P.	log I.P.
$10^{-3}$	3868	161.2	0.16	$\bar{0}.80$
$10^{-4}$	952	39.7	0.40	$\bar{0}.40$
$10^{-5}$	196	8.17	0.82	$\bar{0}.09$
$10^{-6}$	50	2.08	2.08	0.32
$10^{-7}$	14	0.583	5.83	0.77
$10^{-8}$	0	-	-	-

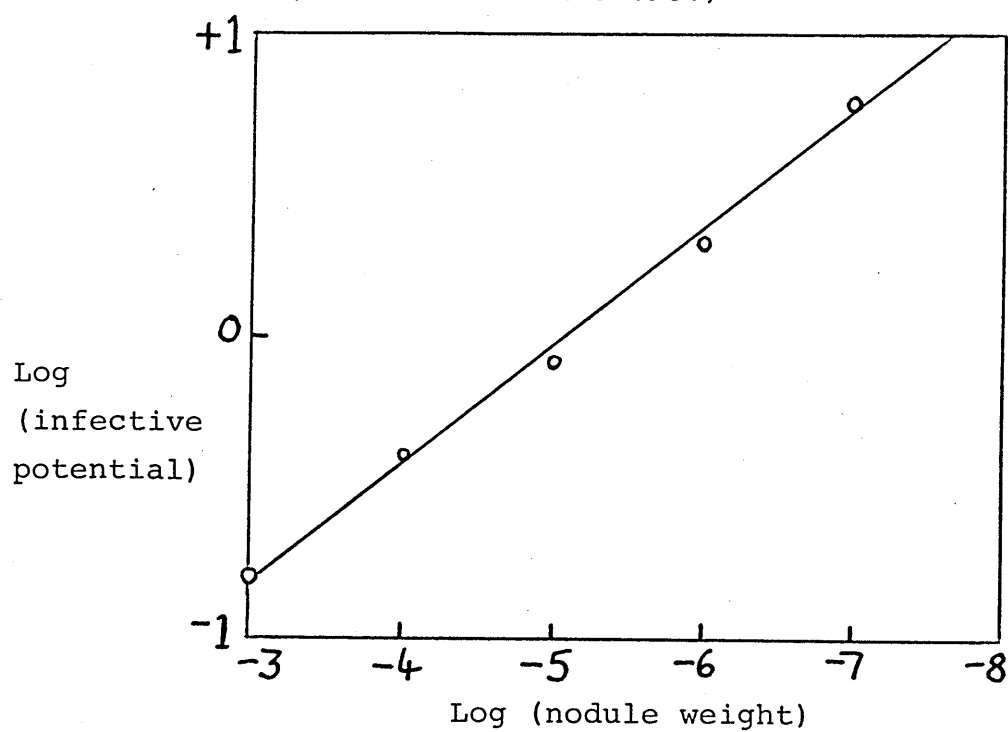
W = weight of dry nodule powder present in the homogenate - in grams

$N_t$  = total nodule number (for 24 samples)

$N_m$  = mean nodule number =  $\frac{N_t}{24}$

I.P. = infective potential =  $N_m \times W$   
(particles/g dry nodule powder)

Graph 1.46 Variation in infective potential with different concentrations of homogenate (data from Oremus 1980)



mean infective potential =  $2.29 \times 10^6$

### 1.8.2 Changes in I.P. storage

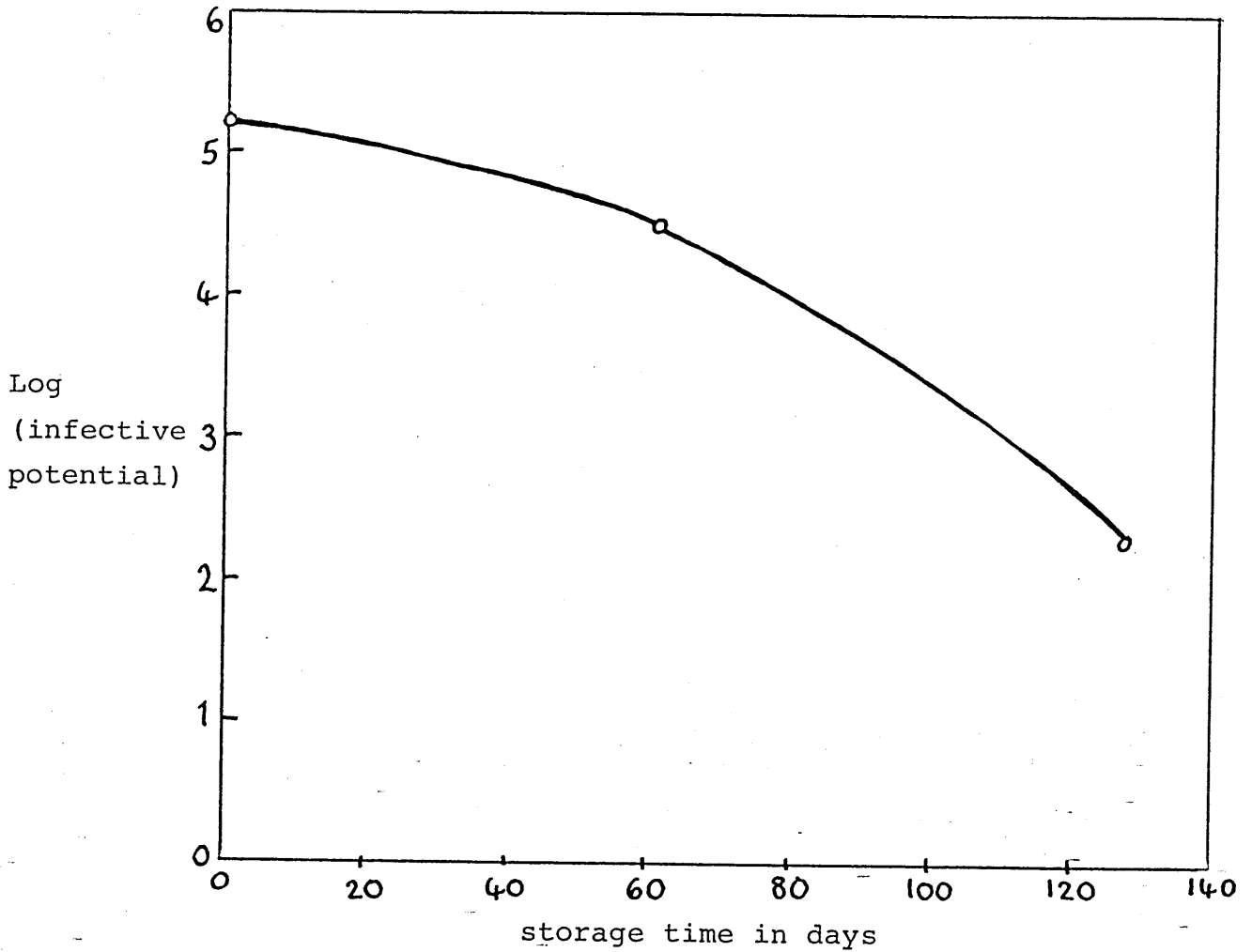
Having established the validity of the assay methods Oremus turned to the question of whether the homogenates or dried nodule powders were stable over a period of time. He presented his results in tabular form (his Tables 2 & 3 p. 128) and these are reproduced below in an abbreviated form, but including log values. (Table 1.24)

The results are given in a graphical display and it is clear that I.P. declines markedly with storage time particularly when in aqueous suspension (Graph 1.47 A and B). In any experimental usage it would be necessary to assay the powders before hand to establish the I.P. From the point of view of general infectivity the powders remain a potent source of endophyte and Oremus commented that "air-dried nodule powders can be stored for relatively long periods with maintenance of a high I.P." (p. 129)

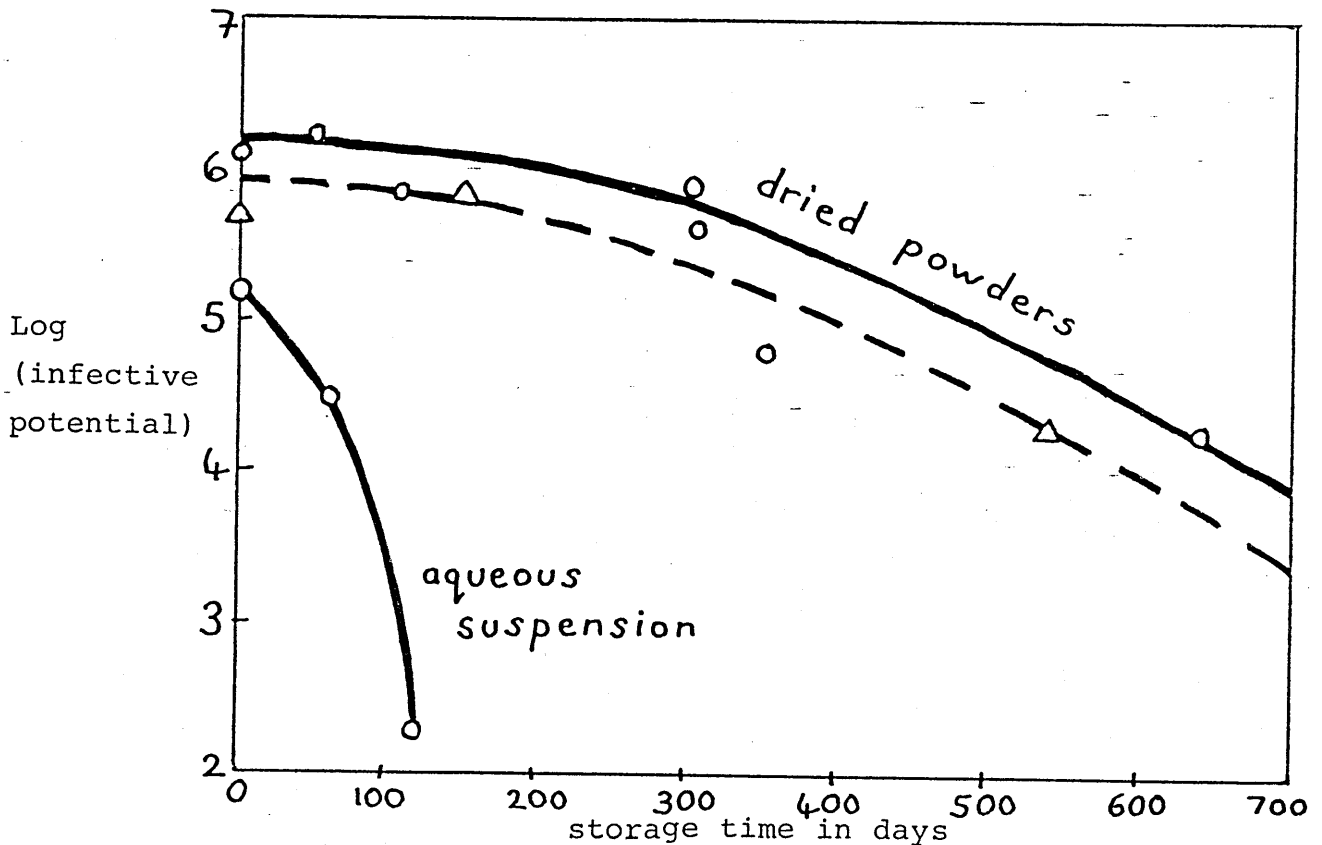
Table 1.24 Variation in infective potential of nodule material with storage time (data from Oremus 1980)

Time - days	I.P.	Log I.P.	Time - d.	I.P.	Log I.P.
0	$1.2 \times 10^6$	6.08	0	$4.9 \times 10^5$	5.69
51	$1.7 \times 10^6$	6.23			
106	$7.0 \times 10^5$	5.85	152	$7.2 \times 10^5$	5.86
302	$8.0 \times 10^5$	5.90			
304	$4.2 \times 10^5$	5.62			
350	$6.1 \times 10^4$	4.79			
638	$2.0 \times 10^4$	4.30	540	$1.9 \times 10^4$	4.28

Graph 1.47A Decline in infective potential of nodular material in aqueous suspension, with storage time. (data from Oremus 1980)



Graph 1.47B Decline in infective potential of nodular material in the form of dried powder, with storage time (data from Oremus 1980)



### 1.8.3 Variation in the infective potential of dune soils

Once the reliability of the endophyte particle assay technique was clearly established Oremus went on to the main part of his investigation concerning the infective potential of natural dune soils. He collected soil samples from four different habitats representing successional stages in the development of *Hippophaë* scrub. "Sterile" *Hippophaë* seedlings were grown in these soil samples, for a period of fourteen weeks and were watered with sterile distilled water. The seedlings were then harvested and nodule formation was estimated by counting the nodule number, and measuring the dry weight of nodules in the different soil samples.

As controls he used a further series of "sterile" seedlings, grown in the range of soil samples, but watered with a sterile, nitrogen-free aqueous culture medium (Hoagland solution). This was a precaution to establish whether absence of various minerals or trace elements in the notoriously poor dune soils might be responsible for the failure of nodulation or seedling survival.

A third series of "sterile" seedlings were grown in the range of soil samples. These were watered with a nodule homogenate concentrated enough to produce maximum nodulation on the basis of earlier experiments. The purpose of this control was to see if any failure to develop nodulation was due to lack of infective endophyte particles, or to some other cause.

Oremus also measured the dry weight of seedlings after harvesting as a useful datum, and he quoted dry nodule weight

as a percentage of dry plant weight in his results. As a final refinement he sampled soils on the surface and at 30-60 cm depth in three of the successional stages (excluding the sea-beach which can be assumed to be homogeneous). This was another wise precaution given the marked difference between surface and sub-surface conditions established by his earlier study (OREMUS 1979) and others (VASCHENKO 1973).

These experiments constitute a four-parameter situation which can be summarised thus:

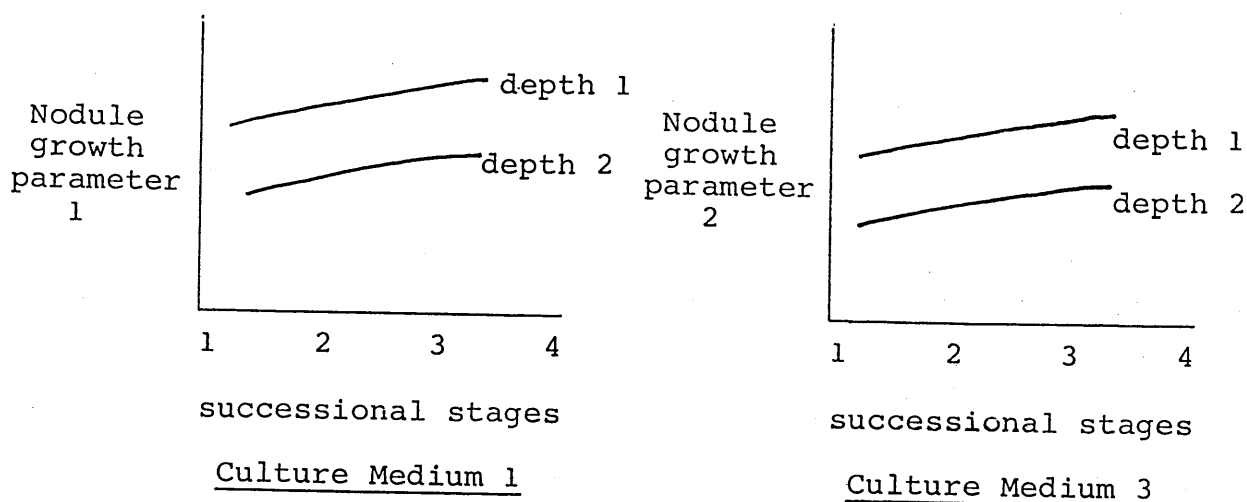
Hippophaë successional stages (4) x culture media (3) x soil depth (2) x nodule growth (2)

The numbers in brackets indicate the sub-divisions for each parameter. There are various ways in which these parameters can be presented but the arrangement shown in Fig. 1.6 is clear and intelligible. The two figures indicate the first and last graphs in a series of six. (Two growth parameters x three culture media).

The number of graphs is increased beyond six by taking other criteria of nodule growth. Oremus only measured two ( $N_p$ , nodule number, and  $W_p$ , dry nodule weight) but others can be derived by calculation e.g. nodulation as a percentage of dry weight of plant,  $\frac{W_p}{P_p} \times 100 = W\%$  or the mean nodule size

$$\frac{W_p}{N_p} = W_m$$

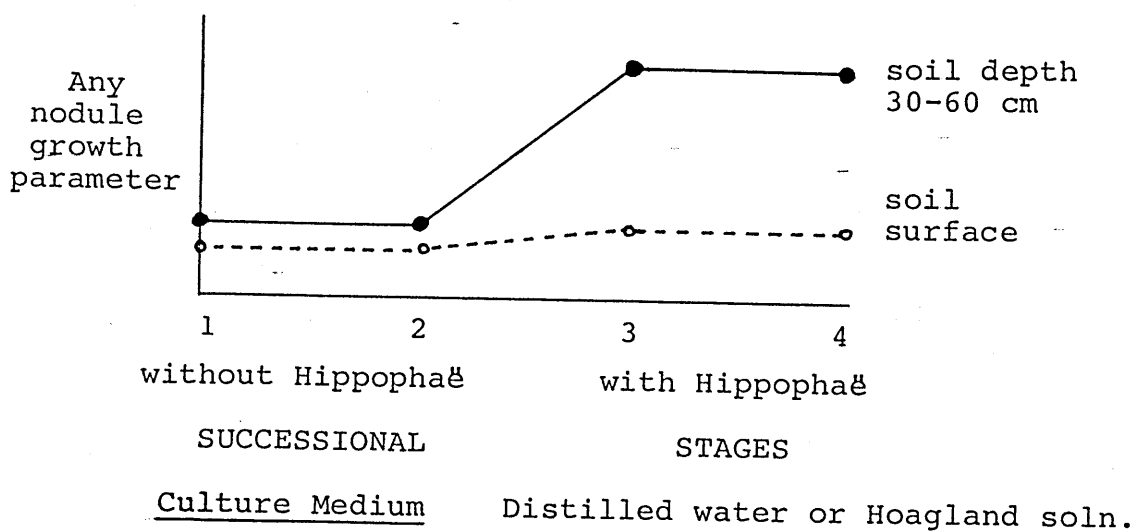
Figure 1.6 Four-parameter display



Using this arrangement Figure 1.7 shows the expected results given four basic assumptions:

- (i) that most nodules occur well below the surface,
- (ii) that mineral nutrient lack is not a critical factor,
- (iii) that each of the growth parameters yield very similar results,
- (iv) that when *Hippophaë* is present in a particular successional stage a soil sample will produce maximum nodulation.

Figure 1.7 Expected results - natural dune soil



Oremus gave all the data from these experiments very fully, including growth parameters as means, with standard error in each case. (his Table 4, p. 129) He again limited himself to a discussion of the significance of the results within the text of his paper. Given the intricate, logical basis of these experiments it is worth giving the results in graphical form using the structure discussed above.

The data from Oremus' Table 4 have been simplified and rearranged in my Table 1.25 to facilitate graphical display. An additional column giving mean nodule weight has been added. (Wm)

Table 1.25 Nodulation and growth of Hippophaë seedlings in dune soil samples (Data from Oremus 1980)

1. Culture medium - distilled water

Habitat	Depth	Np	Wp	Pp	W%	Wm
S	surface	20	43	1.24	4.3	2.15
A	surface	4	22	0.68	3.9	5.50
	deep	17	27	0.99	2.9	1.59
HN	surface	19	66	1.48	4.6	3.47
	deep	307	105	2.27	4.5	0.34
HNN	surface	181	78	2.69	2.9	0.43
	deep	30	39	1.51	2.5	1.30



## 2. Culture medium - nitrogen-free Hoagland soln.

Habitat	Depth	Np	Wp	Pp	W%	Nm
S	s	16	29	0.97	5.6	1.81
A	s d	9 45	15 22	0.71 0.79	2.0 3.1	1.67 0.49
HN	s d	10 330	84 112	1.33 3.51	5.9 3.1	8.40 0.34
HNN	s d	81 33	75 70	2.37 2.16	3.0 3.6	0.93 2.12

## 3. Culture medium - N-free Hoagland + nodule homog.

Habitat	Depth	Np	Wp	Pp	W%	Nm
S	s	303	264	3.28	7.5	0.87
A	s d	205 375	137 125	2.98 2.61	4.3 4.5	0.67 0.33
HN	s d	16 283	40 102	1.05 1.43	3.9 7.5	2.50 0.36
HNN	s d	58 47	56 49	1.79 1.21	3.3 4.2	0.97 1.04

## Key to Table 1.25

S = sandy beach  
A = yellow *Ammophila* dune } No *Hippophaë*

HN = fixed dune - young *Hippophaë* suckers nodulated

HNN = fixed dune - young *Hippophaë* suckers - non-nodulated

Np = number of nodules per plant pot - mean\*

Wp = dry weight of nodules per pot - in mg - mean\*

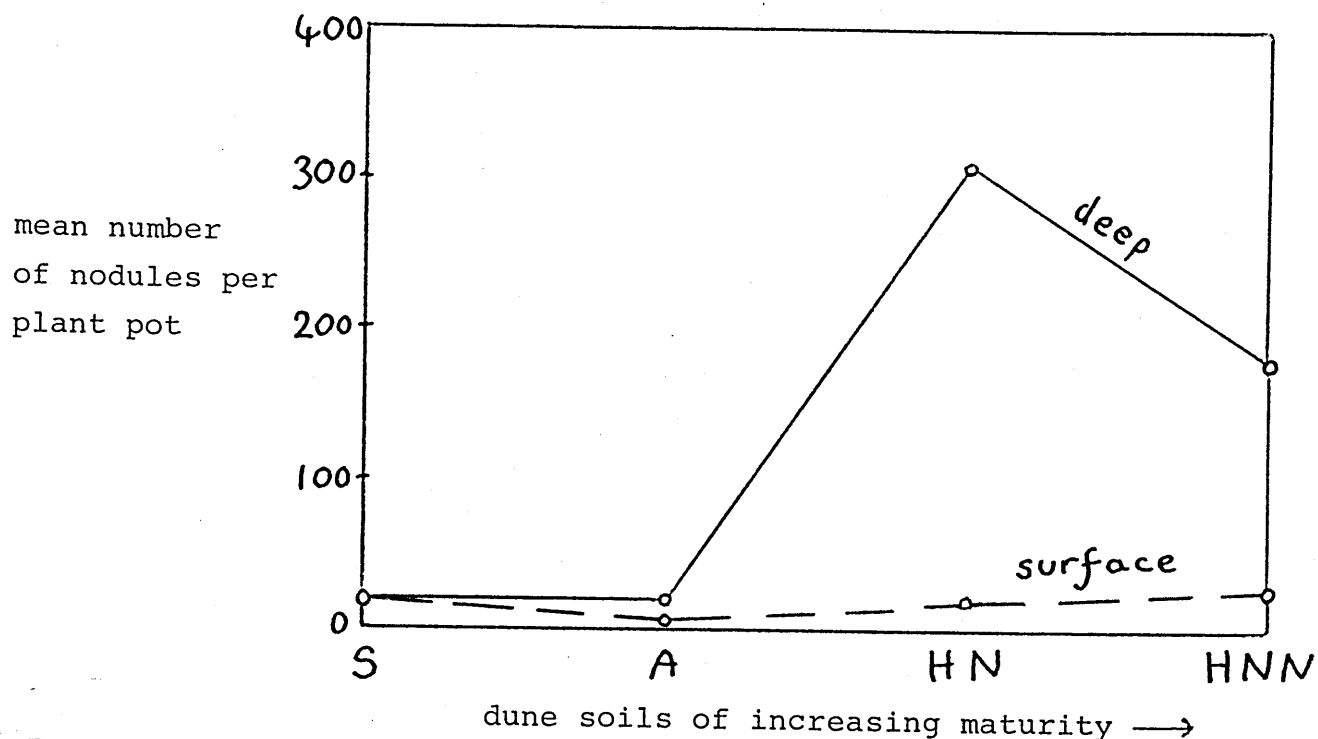
Pp = dry weight of plants per pot - in g - mean\*

\* mean of three replicates

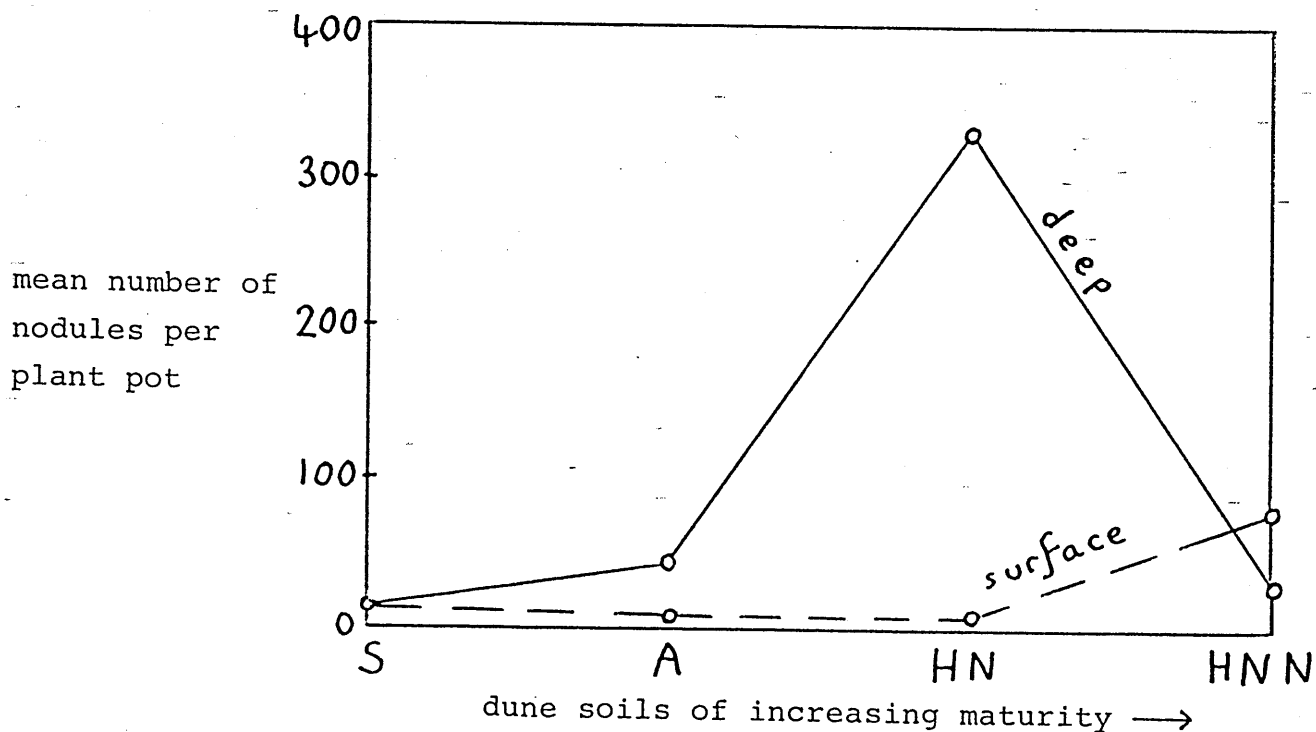
W% = nodule dry weight as a percentage of plant dry weight

Wm = mean nodule weight - in mg  $\left(\frac{Wp}{Np}\right)$

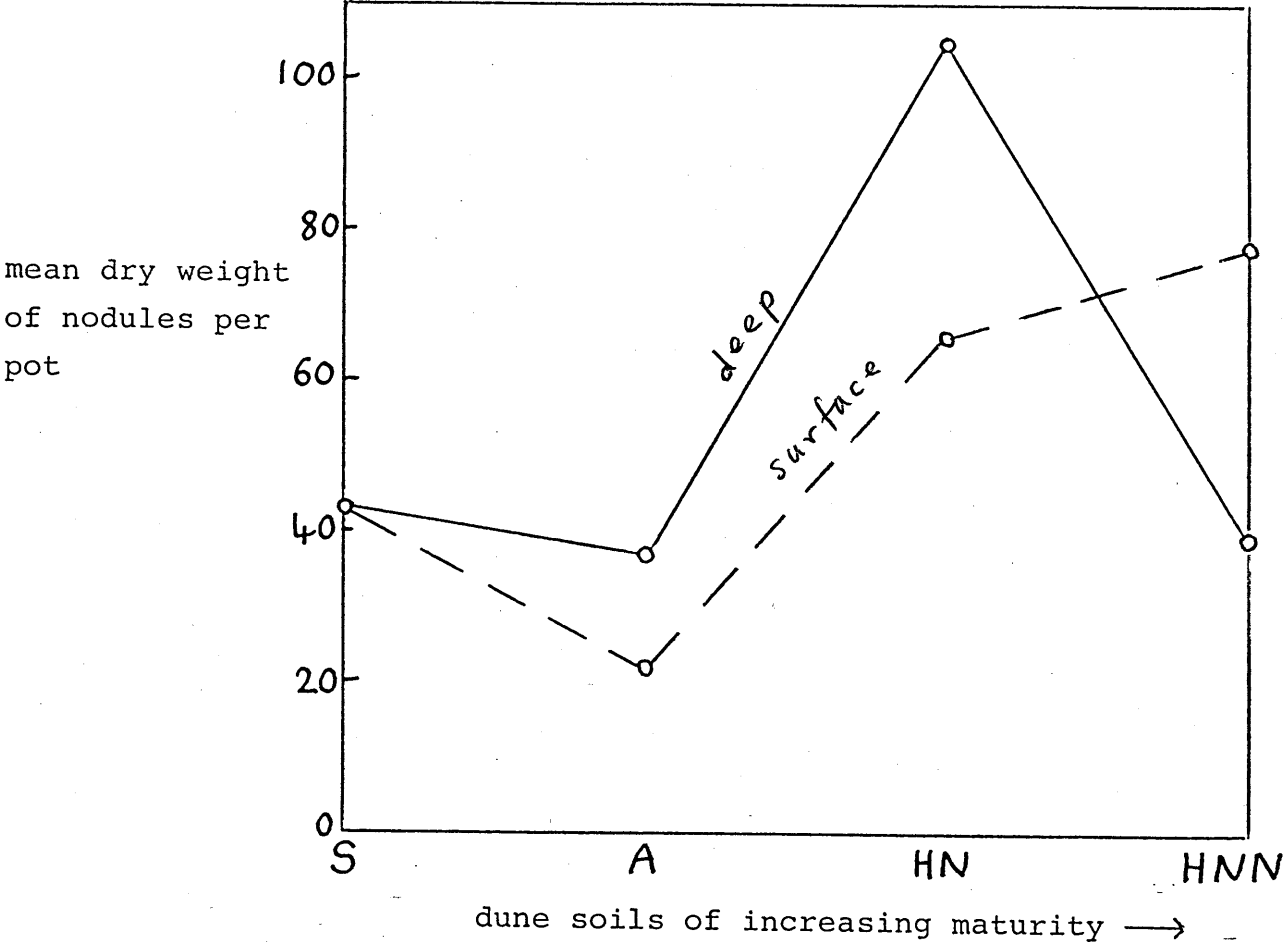
Graph 1.48 Nodule frequency in *Hippophaë* seedlings grown in a range of dune soils - distilled water irrigation. (data from Oremus 1980)



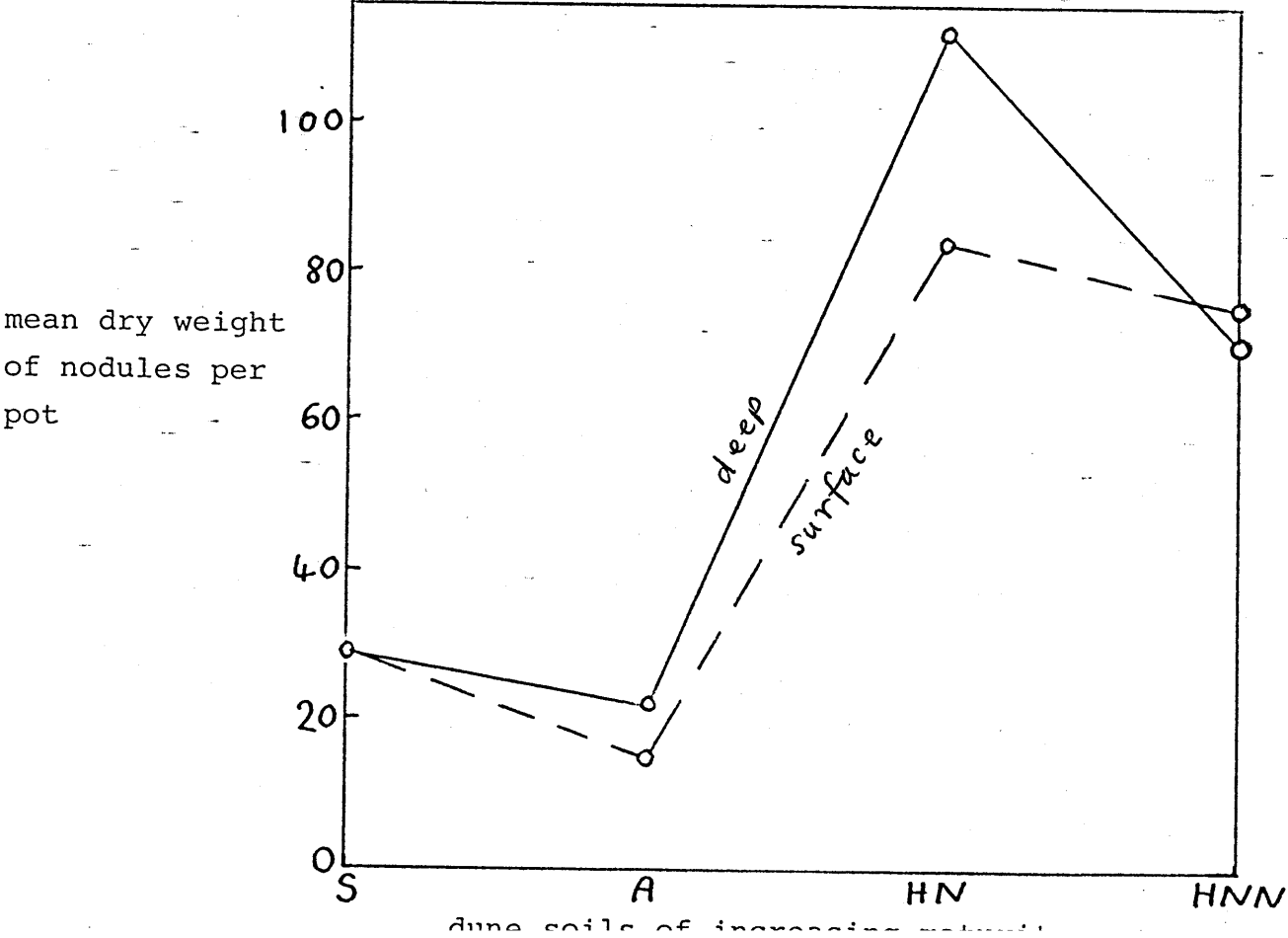
Graph 1.49 Nodule frequency in *Hippophaë* seedlings grown in a range of dune soils - Hoagland solution irrigation. (data from Oremus 1980)



Graph 1.50 Increase in nodule weight, in Hippophaë seedlings grown in a range of dune soils - distilled water irrigation (data from Oremus 1980)



Graph 1.51 Increase in nodule weight, in Hippophaë seedlings grown in a range of dune soils - Hoagland solution irrigation (data from Oremus 1980)

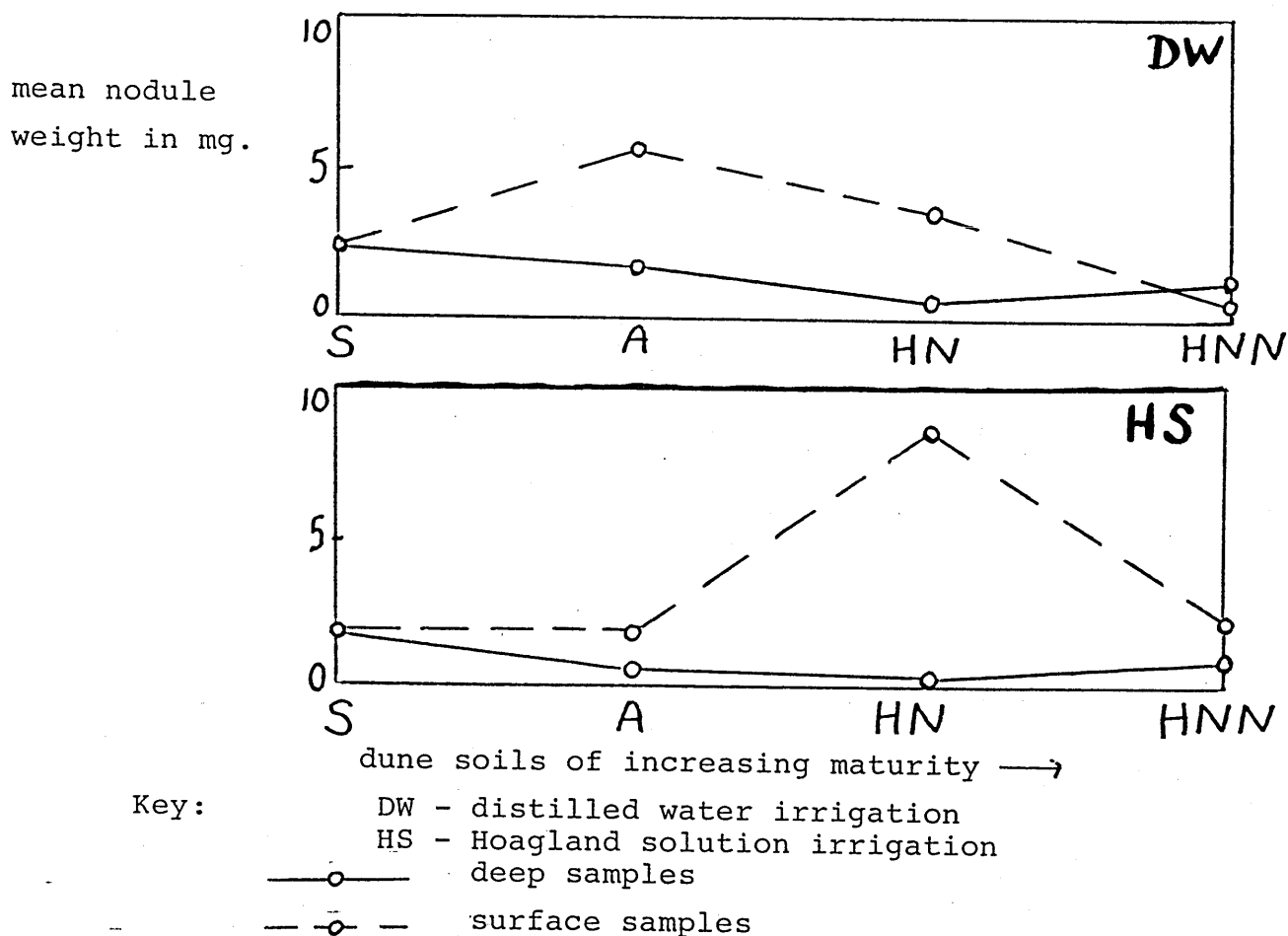


The expected results are shown in Fig. 1.7 and the observed results appear in Graphs 1.48, to 1.49 for the nodule-frequency parameter  $N_p$ . A low level of nodulation in seedlings grown in surface soils is evident, and a similar situation is found in the Hippophaë-free soils. A high level of nodulation is associated with deep soils from Hippophaë habitats, although the older successional stage shows less nodulation. As  $N_p$  is the number of nodules observed it is the best guide to the number of particles of endophyte present in the soil.

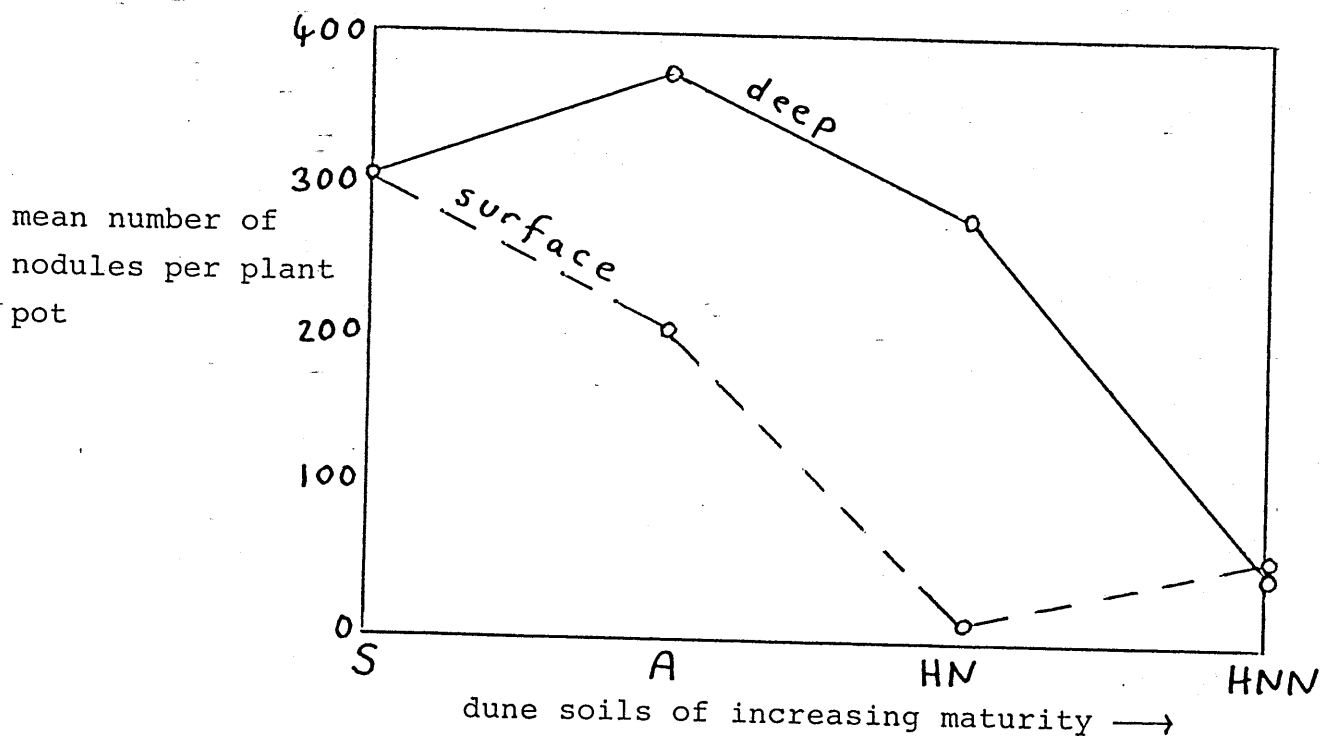
The actual development of nodules on infected roots is best indicated by  $W_p$  the dry weight of nodules, as shown in Graphs 1.50 and 1.51. Again non-Hippophaë soils yield low levels of nodule growth, and Hippophaë soils show higher levels, but later successional stages rather less. A striking difference is seen in the seedlings grown in surface soils, which though poorly - nodulated in terms of numbers show almost as much nodule-weight as those grown in deeper soils.

It seems likely that in surface soils infected sites on the roots of the seedlings are fewer, but the nodules which develop grow to a larger size "in compensation" so that the amount of N-fixing tissue is much the same as that in seedlings grown on deeper soils. However it is curious that a similar increase in nodule size is not observable in the seedlings grown in non-Hippophaë soils. In Graph 1.52 the mean nodule-weight  $W_m$  is shown and it is clear that the seedlings in surface soils have larger nodules, and in some cases the nodule size increases in those grown in non-Hippophaë soils. It seems likely that there is a

Graph 1.52 Nodule weight in Hippophaë seedlings within a range of dune soils, given two different irrigation treatments. (data from Oremus 1980) 143



Graph 1.53 Nodule number of Hippophaë seedlings in a range of dune soils augmented by nodule homogenate (data from Oremus 1980)



considerable amount of variation in this situation, and a larger sample size would be necessary to establish the validity of these apparent trends.

Three, of the four original assumptions are borne out by these experiments. (i) Nodules developed best in seedlings grown in deeper soils, because in the field few nodules occur naturally in the surface layers. (ii) Mineral nutrient lack does not seem to be critical as the difference between the results obtained by using distilled water or the Hoagland solution are negligible. (iv) The presence of Hippophaë in a habitat does facilitate the development of seedling nodulation, probably because of the infective potential of dead nodule material in the soil.

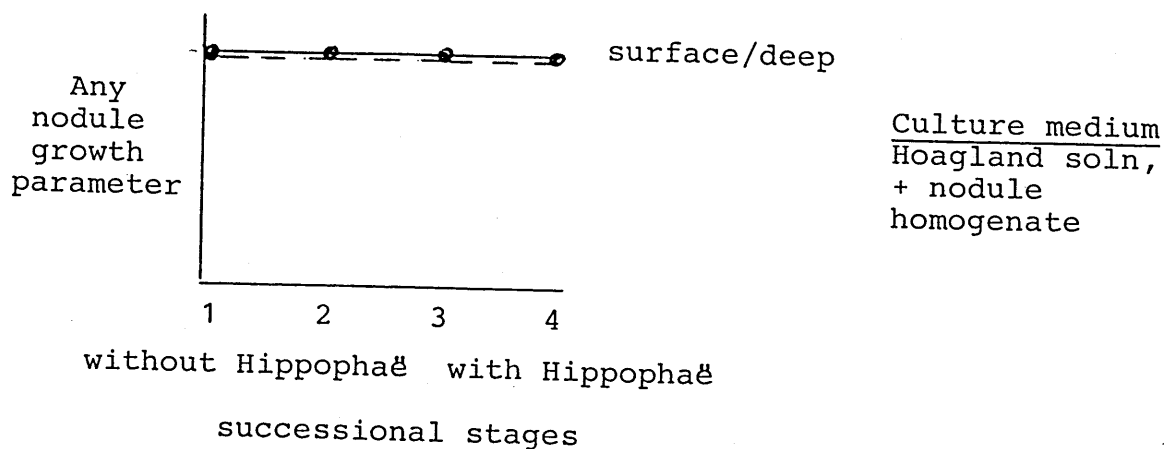
In contrast (iii) the growth parameters did not yield entirely similar results, but there was enough to confirm the first three assumptions.

The third set of experiments involved growing Hippophaë seedlings in a range of soil samples from different successional stages, and watering them with Hoagland culture solution and sufficient nodular homogenate to induce maximum nodulation.

The expected results are indicated in Fig. 1.8 which includes all four parameters. The assumptions in this case are two:

- (i) that nodule growth parameters yield similar nature
- (ii) that different habitats yield soils of similar nature.

Figure 1.8 Expected results - nodule augmented dune soils

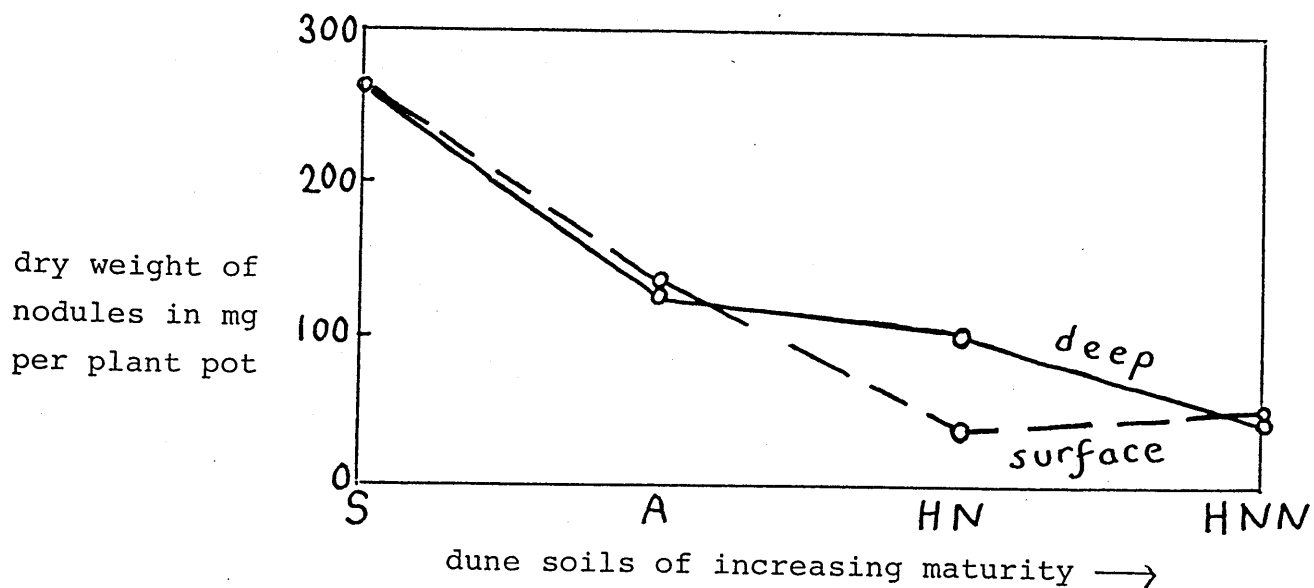


The observed results are taken from Table 1.25 and displayed in Graphs 1.53 and 1.54. The nodule numbers far from being high and constant, decline with the successional stage, and the decline is more marked in the surface soils. However the graph of dry nodule weight shows a more regular decline with successional age, and the difference between surface and deep soils is negligible. The difference in mean nodule size between surface and deep soil samples is small, except for one case (Graph 1.55).

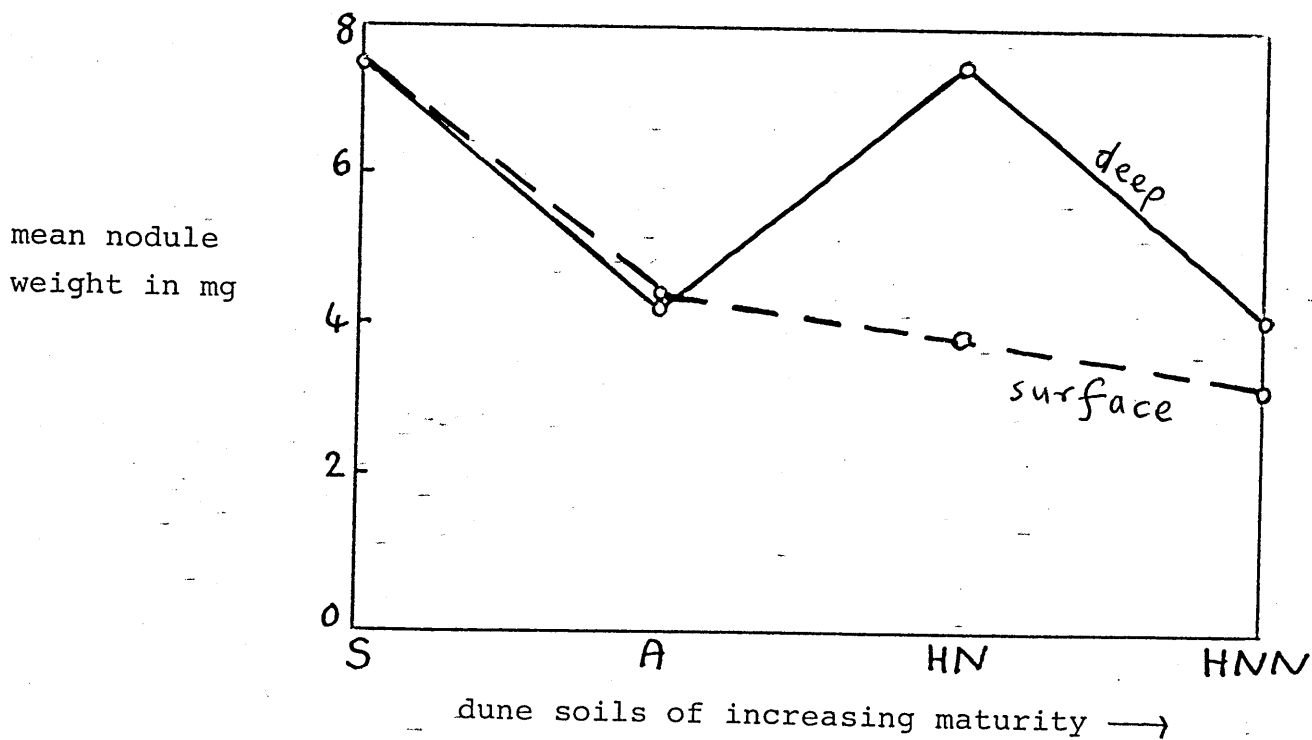
The first assumption is borne out in that the growth parameters are similar, but the second is not. For some reason, nodulation in older successional stage soils is significantly lower than in younger stages. As Oremus comments "the addition of endophyte did not enhance nodulation, which indicates that other factors than the endophyte content must be responsible for the low level of nodulation" (p. 130)

Oremus concluded "that there are significant differences between the I.P. of different soil samples." and "since the differences in the I.P. values of soil samples might be

Graph 1.54 Nodule weight of *Hippophaë* seedlings in a range of dune soils augmented by nodule homogenate (data from Oremus 1980)



Graph 1.55 Nodule size in *Hippophaë* seedlings in a range of dune soils augmented by nodule homogenate (data from Oremus 1980)





related to the stage of succession of the *H. rhanmoides* scrub" he carried out a more detailed study (p. 130).

#### 1.8.4 Infective potential at successional extremes

He selected two areas which formed the extreme ends of the *Hippophaë* succession. AH contained young plants with and without suckers, and HP contained scrub at the post-optimal stage. Fifteen soil samples were taken from each site, and endophyte-free seedlings were planted in each type; one series were untreated, and a parallel series were grown and treated with a nodule homogenate solution. No nutrients were used. After the growth period, Oremus measured the nodule number per pot ( $N_p$ ) and the shoot biomass per pot ( $S_p$ ) as fresh weight in g.

In this particular set of experiments Oremus made use of a series of bar charts with mean and standard error displayed. (his Figs. 1,2 and 3). He did not publish the raw data but these have been obtained from the bar charts and are given in Table 1.26.

The key question is whether the soils with endophyte added produced greater nodulation in *Hippophaë* seedlings than did soils without added endophyte. The data from Table 1.26 are presented as a scatter diagram for the fifteen soil samples, using the nodule number  $N_p$  as coordinates, when the value of  $N_p$  for endophyte-added soil, is the same as for endophyte-free soil, the soil sample appears on the diagonal, in Graphs 1.56 and 1.57.

This presentation shows soil samples to the right of the

diagonal as exhibiting a higher level of nodulation in the endophyte-treated portions of the sample.

In Graph 1.56 it is clear that the majority of points lie to the right of the diagonal while in Graph 1.57 the points straddle the diagonal. So the addition of endophyte to AH (young succession) soil brings increased seedling nodulation, while its addition to PH (post-optimal succession) soil does not.

Table 1.26 Variations in nodule development in Hippophaë seedlings grown in dune soil with and without endophyte (data from Oremus 1980)

sample	Np - AH		Np - PH		Sp - AH	
	O	E	O	E	O	E
1	280	260	275	215	4.1	4.0
2	40	135	135	140	2.6	3.4
3	560	445	100	120	4.3	3.8
4	270	255	170	160	3.4	3.5
5	345	250	215	155	4.4	4.3
6	325	240	190	240	4.1	4.8
7	255	435	220	210	4.0	4.3
8	50	110	110	95	2.5	3.5
9	50	165	150	195	2.3	3.7
10	55	370	340	250	2.8	3.6
11	180	200	350	395	4.1	4.1
12	155	215	120	130	4.2	4.3
13	710	1000	190	240	5.5	5.2
14	55	155	50	60	2.8	4.0
15	20	105	40	40	2.1	3.8
Total Mean						

Np - mean nodule number per pot

Sp - mean shoot biomass per pot in g fresh weight

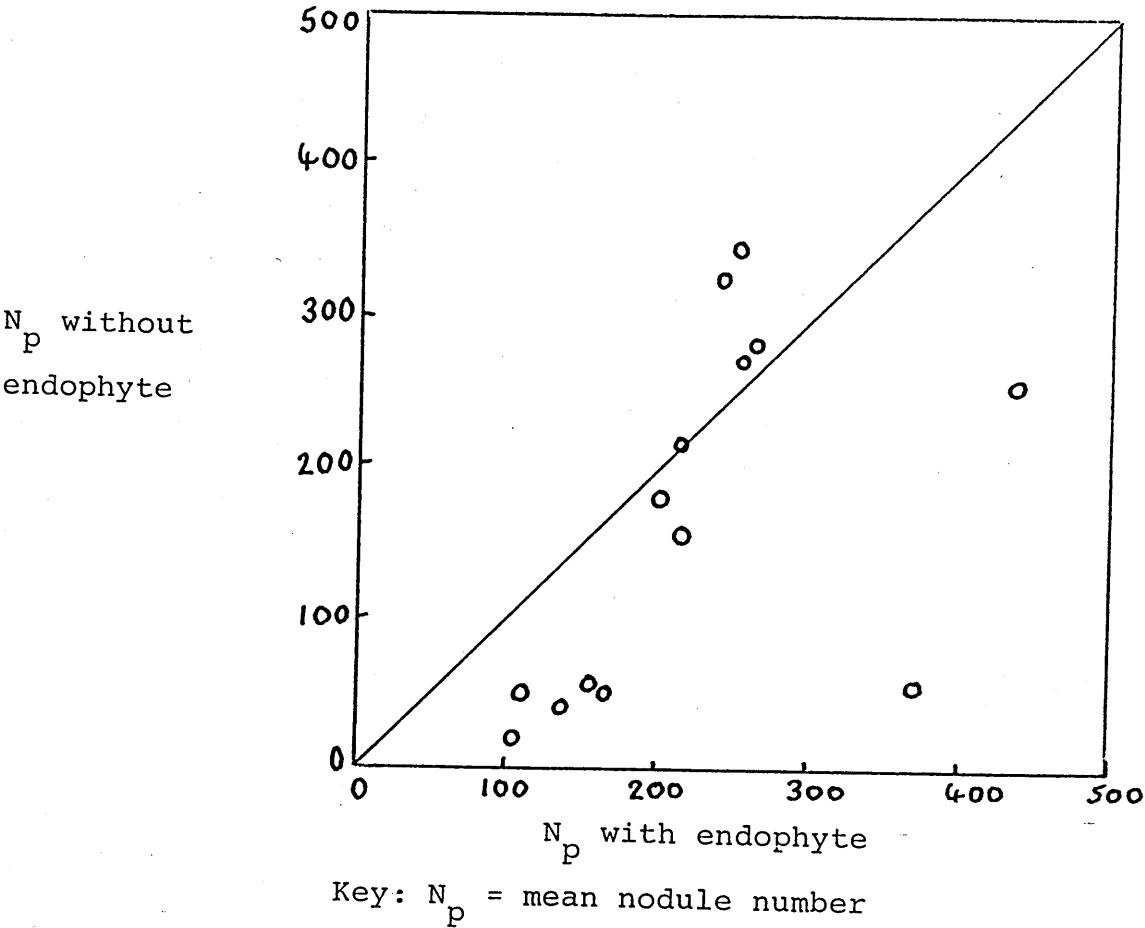
O - no endophyte added E - with endophyte added

AH - early Hippophaë stage

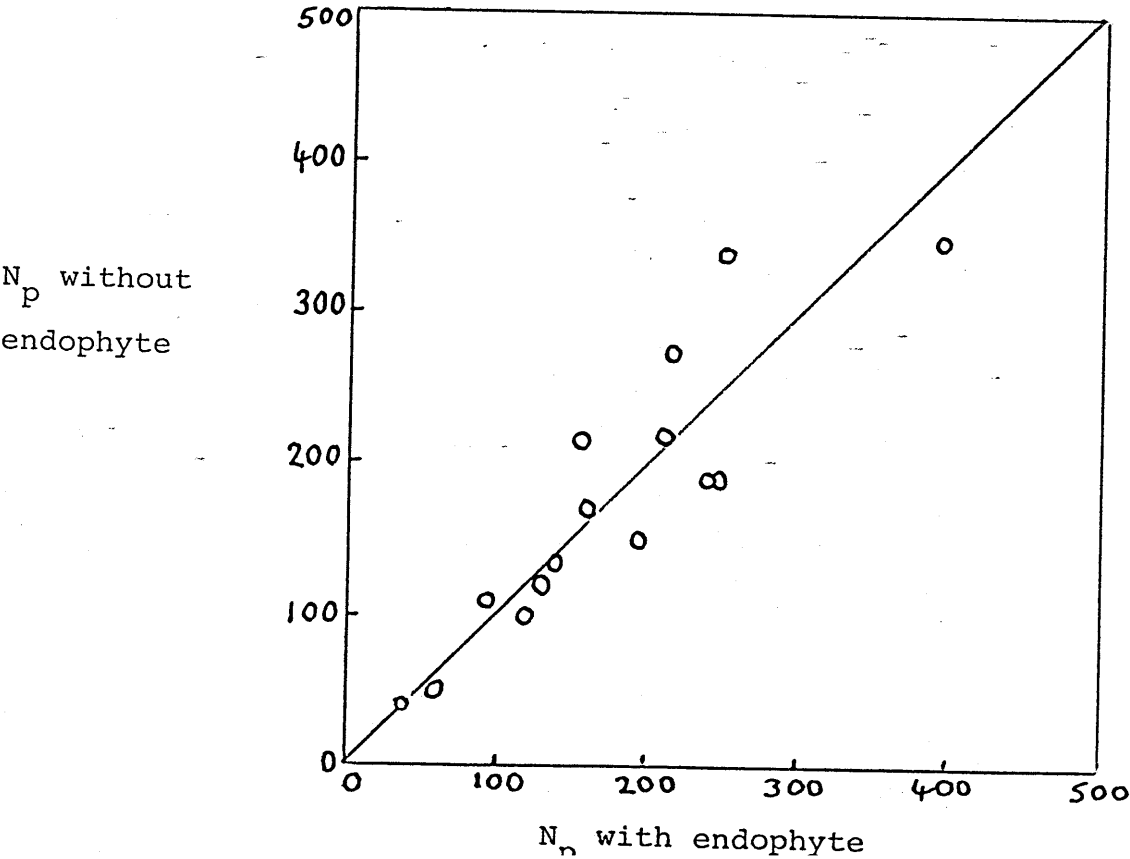
PH - Post-optimal Hippophaë stage

Comparison of nodule development in Hippophaë seedlings grown either with, or without the addition of endophyte; in two types of dune soil AH and PH. (data from Oremus 1980) - Graphs 1.56 and 1.57 146

Graph 1.56 Soil - AH early Hippophaë stage



Graph 1.57 Soil PH - post-optimal Hippophaë stage



Oremus commented that "the numbers of nodules formed in uninoculated soil samples ... are lower than those in the others" and he concluded that "addition of endophyte ... led to a significant increase in the number of nodules formed, and the shoot weight" (p. 130).

Graphs 1.58 and 1.59 show the relationship between nodulation and shoot weight. Where no endophyte has been added, as might be expected, there is a simple relationship; the plants with the greatest nodulation have the greatest amount of shoot growth. (Graph 1.58) In samples where endophyte was added the relationship is much less clear (Graph 1.59). The shoot growth appears to be more or less constant, despite the range in nodulation observed. There is a considerable amount of variation in the results, and a larger sample size would be needed to draw conclusions with greater statistical reliability.

#### 1.8.5 Inhibition of nodulation

The most important discovery made by Oremus, in this series of experiments is that the soil of old successional stages of *Hippophaë* appears to actively inhibit nodulation of seedlings even when infective endophyte particles are added. This could not have been predicted from earlier studies. The value of the elaborate experiments to standardise homogenates and powders is clear, as the significance of the results on inhibition of nodulation beyond question.

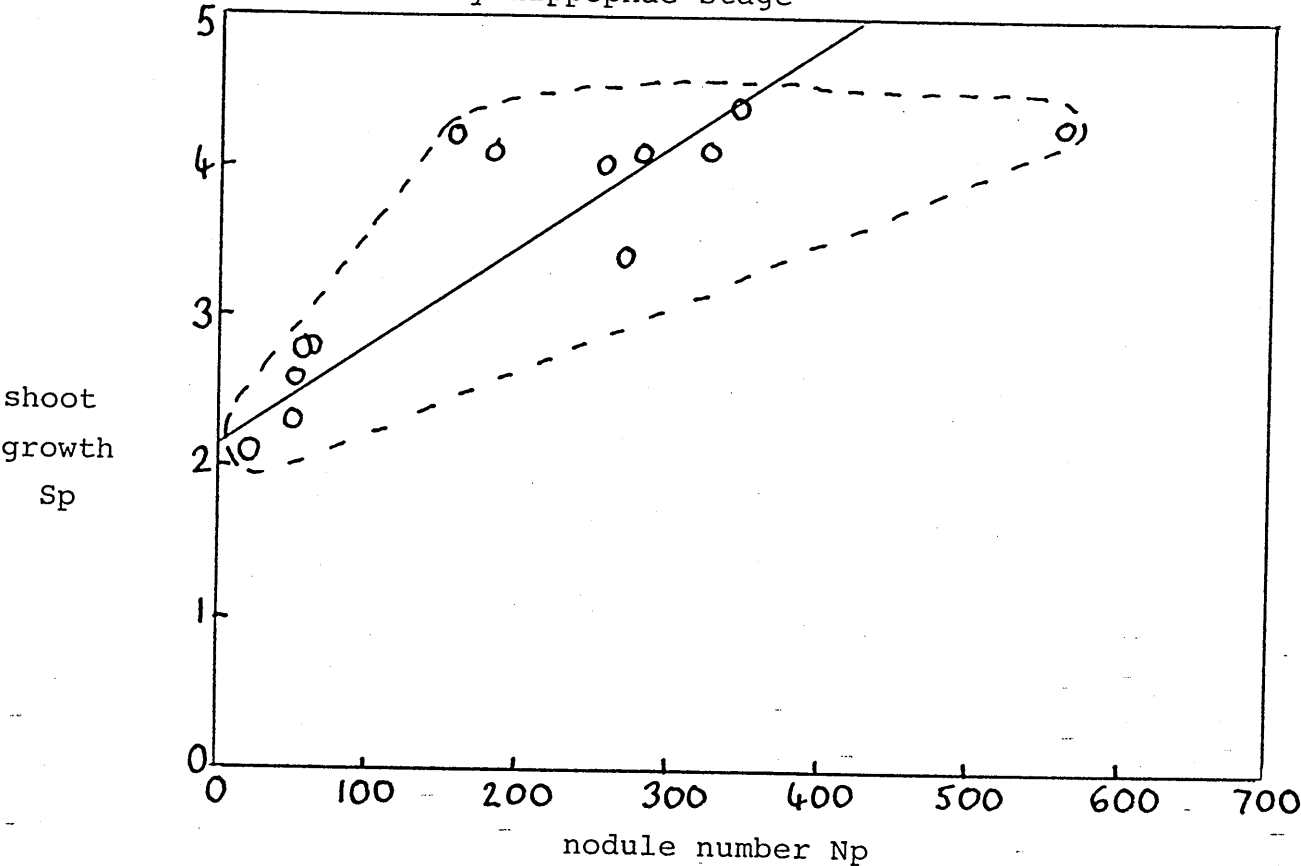
In discussing his results, Oremus noted briefly that not only "secondary environmental factors inhibited nodulation" but also that nodules decayed much faster, and the roots were

(data from Oremus 1980) - Graphs 1.58 and 1.59

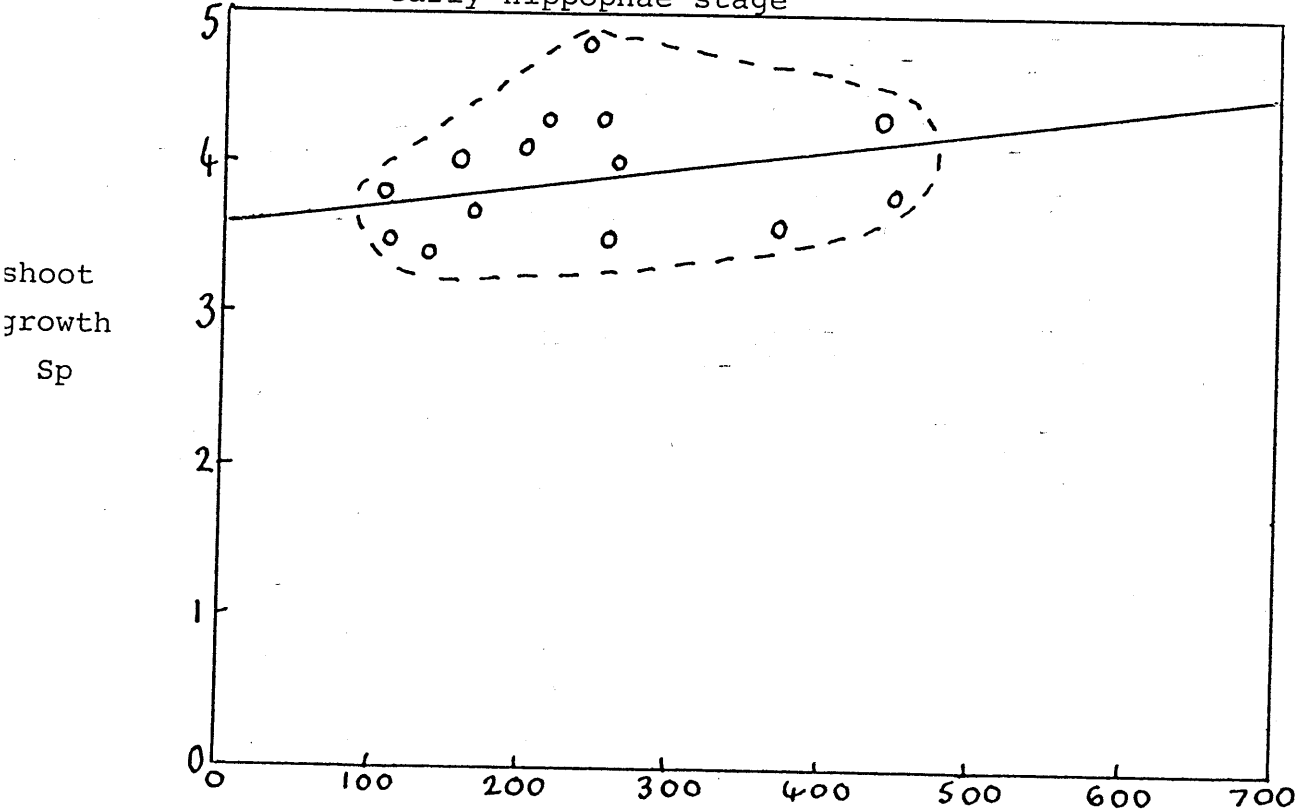
KEY Sp = mean shoot biomass per pot in g fresh weight

Np = mean nodule number per pot

Graph 1.58 Without the addition of endophyte, soil AH - early Hippophaë stage



Graph 1.59 With the addition of endophyte, soil AH - early Hippophaë stage



brown and lacking a well-developed ectomycorrhiza.

He briefly noted four possible explanations of the inhibition effect all of which had been observed by other workers.

(i) Pathogens

"Low nodulation of test plants grown in soil from post-optimum Hippophaë scrubs might be due to interference with (? by) pathogenic microorganisms." He pointed out that antagonism between nodule-formers and other microorganisms had been observed in clover by Hely Bergersen and Brockwell.

(ii) Absence of 'assistant' microorganisms

As the need for 'assisting' microorganisms had been shown to be necessary by Knowlton, Berry and Torrey in the nodulation of Alder it might also be that the "absence of 'assisting' microorganisms ... might play a role in the infection process" in Hippophaë.

(iii) Soil nitrogen

Although "the concentration (of nitrogen) at which nodulation is depressed when plants are growing in the soil is not known..." there is evidence from Bond and his co-workers that the nodulation of Hippophaë in liquid cultures is inhibited by nitrogen as nitrate or ammonium at the levels measured in soil solutions by Oremus. This was 18 ppm N as  $\text{NO}_3^-$  and 4.5 ppm N as  $\text{NH}_4^+$ .

In his Table 5 (p. 133) he quotes the organic matter and total N for the two sites AH and PH. He does not give data on  $\text{NO}_3^-$  or  $\text{NH}_4^+$  levels.

(iv) Soil particle size

He notes that according to Ledwood and Shimwell the growth of Hippophaë is best on deep, loose sand and given that he found "small differences in particle size between the sand of sites AH and HP" it might be that this could affect the differences in nodulation observed between the two sites.

The study was concerned primarily to establish a relationship between the distribution of the endophyte and the extent of nodulation. While making his investigations Oremus obtained evidence of nodulation inhibition. In conclusion he remarks that "further studies are needed to elucidate the significance of these factors in the nodulation process in the field" (p. 137). These experiments were directly relevant to the hitherto unexplained cycle of growth, maturation and decay of Hippophaë rhamnoides. Having identified the significant factors he followed this up with a further study which offers a simple solution of the mystery.

1.9 Causal factors in the inhibition of root-nodulation in Hippophaë at mature successional stages.

A carefully designed series of experiments were carried out to try to discover the basis of the inhibition of nodulation. These involved soil sterilization and seedling culture. (OREMUS and OTTEN 1981) Much of the experimental technique was based on that established in earlier work, and discussed in previous pages.

Soils were collected from the two extremes of the Hippophaë succession, AH the youngest, and HP the post-optimum stage. Some samples were sterilized by ignition to 900 °C, and others by gamma irradiation. Other samples were left untreated as controls. A further series were "inoculated" by mixing 20 g of an unsterilized soil with a bulk of 1530 g of the main sample. Each sample was further inoculated with 20 mg of a nodule homogenate sufficient to ensure maximum root-nodulation, on the basis of earlier experiments.

The soil samples were used as culture media for growing "sterile" (non-infected) Hippophaë seedlings, and after harvesting, the extent of nodulation, root and shoot growth were estimated in several different ways.

The data were displayed as the means (and standard error) of six replicates of each type of treatment with a particular soil sample. The authors appear to have made some statistical analysis of the raw data, as on p. 320 they refer to a correlation of low nodulation with low plant dry weight ( $P = 0.05$ ) but none of this analysis is given in the published paper.



It is difficult to subject the data to statistical analysis because only means are available. Although the results are numerical the actual analysis given by the authors is of a qualitative nature, such that 'datum X is bigger than datum Y', or 'data A and B are not significantly different from each other'. This is primarily because the variables are non-linear (irradiation, inoculation, soil type) and are not amenable to a simple quantitative graphical analysis of the XY type.

Nevertheless it is possible for data in this form to yield a clear picture of the effects of sterilization, and inoculation on the growth and nodulation of seedling plants. In this particular series of experiments the results obtained, though consistent, did not provide simple conclusions. The consistency suggests that the effects were not random, and logical explanations might be found for the results of seedling growth.

However almost every result poses more questions than it answers. The authors state their findings in general terms (pp. 320-322) and discuss them (pp. 327-329) but avoid drawing attention to the many puzzling features of their results. This is not unreasonable as there are no obvious answers to the difficulties and any speculations would be valueless without a further series of comprehensive tests.

As an illustration of these problems I select one example. Nodulation of seedlings grown in the HP soil is much lower than those grown in AH soil. It is about half as much, due we assume to the action of some biological agent which inhibits nodulation, despite the fact that added endophyte

contains plenty of infective particles to start root-nodulation.

When HP soil is sterilized by irradiation or ignition, both the inhibiting agent and the natural endophyte should be destroyed. When fresh endophyte is added as homogenate and also unsterilized soil inoculum we should expect the seedlings to show an increased rate of nodulation, as the effect of the inhibitor is absent. However, in the words of the authors "ignition and irradiation of HP soil had no effect on the number of nodules". Treatments 3 to 7 gave very similar results for HP soils

How may this be explained? Is the inhibitor a chemical agent unaffected by ignition at 900 °C or irradiation? Is there sufficient nitrogen in the HP soil to make root nodulation unnecessary for adequate seedling growth? Is the inhibitor reintroduced with inoculation? Is the crucial process the adequate development of root systems rather than nodules?

To answer any of these questions requires a much fuller experimental analysis. It seems from subsequent experiments that the fourth question is the key one, but an affirmative answer here still leaves a mystery. If root systems are all important why is it that similar young seedlings in AH soils nodulate so vigorously?

The authors' findings, in general terms, are indicated below.

- (i) Plants grew better in AH than HP soils.
- (ii) The number and dry weight of living nodules were

- higher in untreated AH than in untreated HP soils.
- (iii) Dry matter of shoots was higher in HP plants than AH plants.
  - (iv) The number of necrotic nodules was higher in HP soil than AH soil.
  - (v) The number of nodules formed in AH soil was reduced after soil sterilization.
  - (vi) Sterilization of HP soils had no effect on live nodule number.
  - (vii) Sterilization of HP soils reduced the number of necrotic nodules.
  - (viii) Ignition of AH soil reduced seedling dry weights.
  - (ix) Ignition of HP soil had no effect on dry weights.
  - (x) Irradiation of AH and HP soils had no effect on seedling dry weights or nodule dry weights.
  - (xi) Irradiation of HP soil decreased the shoot dry weight.
  - (xii) Inoculation of soils, sterilized or untreated, in almost any combination had no significant effect in any of the growth parameters.
  - (xiii) Inoculation of irradiated AH soil with unsterilized AH soil gave an increase in seedling dry weight and live nodule number. (This was "probably due to the significantly higher fresh weight of these plants at the start of the experiment" p. 321-2).

In short, the nodulation experiments, and measurement of plant, and shoot dry weights, give equivocal answers to the questions posed by sterilizing, and inoculating soil samples from the ends of the Hippophaë succession.

Nitrogen data from soil analyses (Table 1 and Table 4 Oremus & Otten 1981) have been extracted and presented as Table 1.27 below.

Table 1.27 Nitrogen in dune soils (Oremus & Otten 1981)

Soil	Treatment	pH	N tot	s.e
AH	raw	9.24	52	
HP	raw	9.11	132	
AH	untreated		1958	42
HP	untreated		1700	44
AH	irradiated		2123	77
HP	irradiated		2031	73

KEY: raw = soil samples not used for growth experiments, in their natural state

untreated = unsterilized, but used to grow Hippophaë seedlings

irradiated = sterilized and used to grow Hippophaë seedlings

N tot = total Nitrogen in ppm

s.e = standard error of the mean

The level of total nitrogen in the natural soils is significantly different, with nearly three times as much in the HP soil compared with the AH soil, due to the fact that the former is a late successional stage. All four soils used as seedling culture media show greatly enhanced levels of soil nitrogen around twenty times as much, due to nitrogen fixation by nodules and later necrosis. The difference between the four soil types was not significant.

Table 1.28 Sterilization and inoculation of dune soils -  
effect of root structure  
(data - Oremus & Otten 1981)

Treatment	Root Dry Wt mg		Root Length mm	
	AH	HP	AH	HP
1. Unsterilized	137	153	5520	3650
2. Unsterilized For. Inoc.	190	186	6247	2952
3. Sterilized uninoculated	217	177	7560	5620
4. Sterilized Comp. Inoc.	170	238	5810	5924
5. Sterilized For Inoc.	192	230	5779	6236
Soil Type	AH	HP	AH	HP

KEY: For. Inoc. - Foreign Inoculation

inoculated with untreated soil of other  
type

Comp. Inoc. - Comparable Inoculation

inoculated with untreated soil of same  
type

Table 1.29 Nematodes in 200 ml of soil (Oremus & Otten 1981)

Species	AH	HP
Saprophytic nematodes	1160	415
Rotylenchus goodeyi	135	0
Telotylenchus ventralis	25	0
Mascroposthonia sp.	20	0
Hemicychiophora sp.	1	0
Merlinius sp.	0	20
Longidorus sp.	0	110

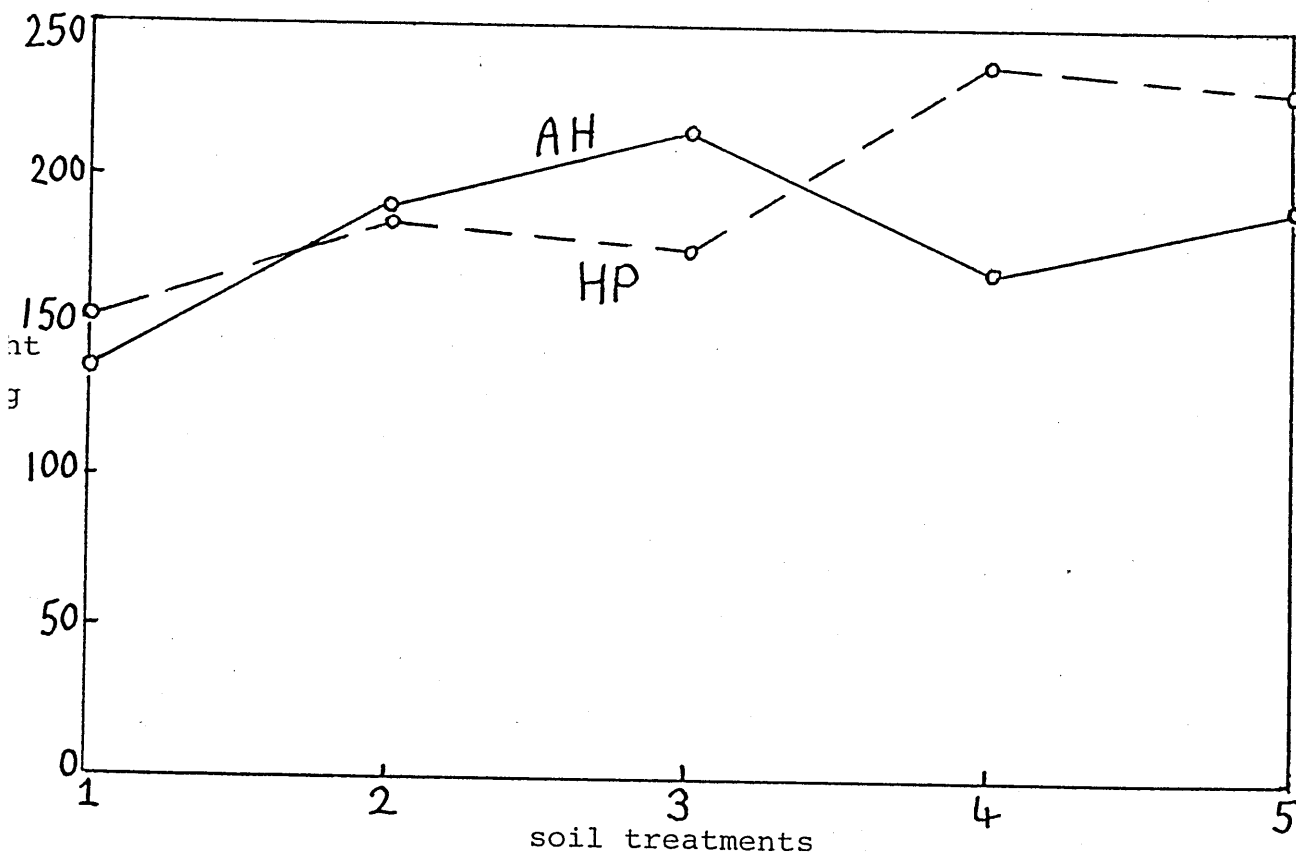
Perhaps the most informative results were those based on the measurement of root length. These are given in Table 1.28 based on Table 5 of Oremus and Otten p.326 and displayed in Graphs 1.60 and 1.61.

The authors commented that "the root weights of plants in all treatments were not significantly different," but noted that the root length of plants in HP soils were lower than those in AH soils, except for treatments 4 & 5 when the two were identical.

Hence it seems that the main effect of "inhibition" is damage to the root system of Hippophaë rather than any effect on the nodulation process. Sterilization enables the roots of seedlings in either type of soil to develop at much the same rate, again irrespective of any nodulation.

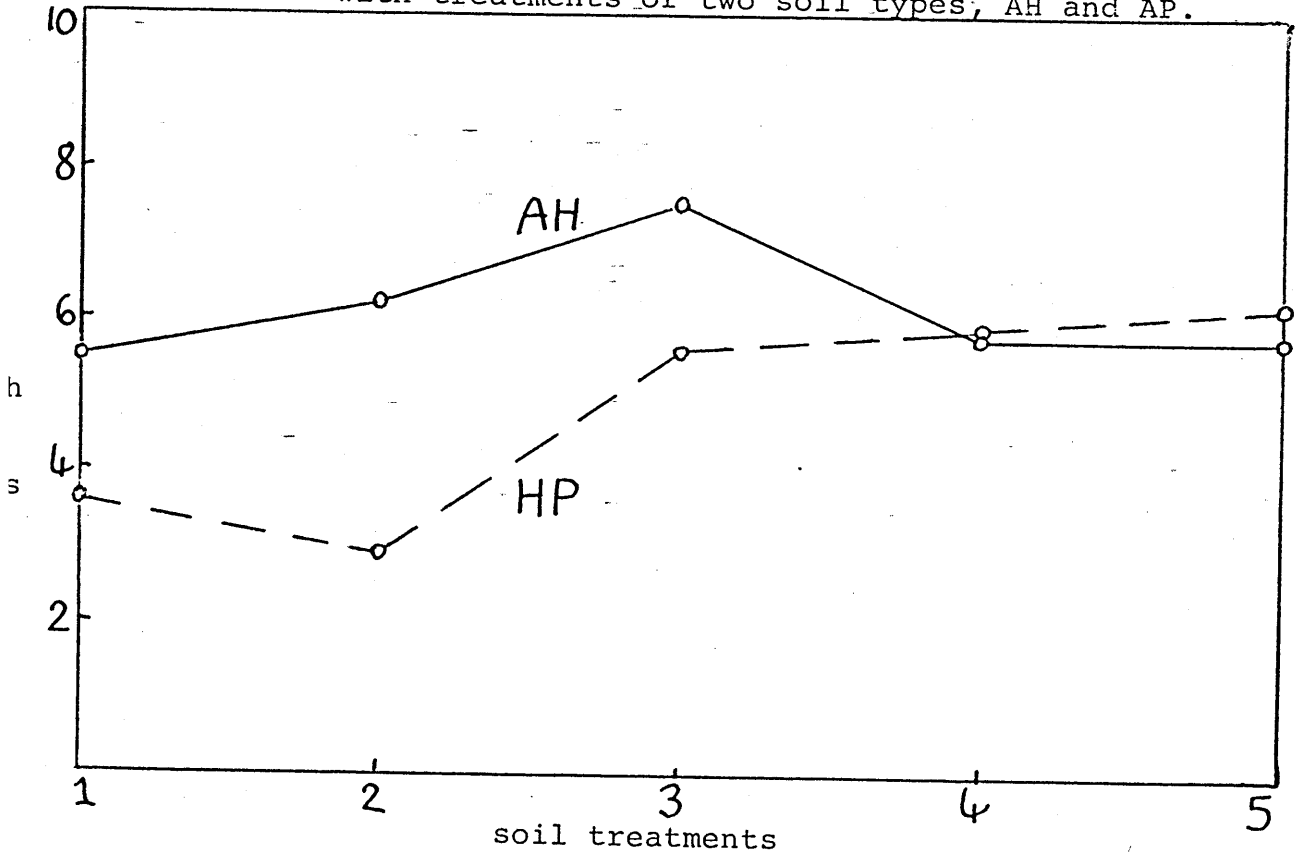
The authors followed up the investigations of root structure by checking for the presence of plant pathogens in AH and HP soils. No plant-pathogenic bacteria were found and the concentrations of fungal plant-pathogens were very small and much the same in both types of soil.

In examining the nematode distributions the authors found three species present in AH but not in HP, a group present in both, but only one, Longidorus, present in HP in great numbers but absent in AH. This, they note, is a species "known to cause in several plant species the kind of root deformations" observed in their Hippophaë seedlings (p. 327). Further, "this relatively large parasitic nematode belongs to a group which transports soil-viruses and may harm root growth directly by inducing deformed, short and swollen roots." (p. 329). See Table 1.29.



Key: 1 = unsterilized, 2 = sterilized, inoculated with untreated soil of another type, 3 = sterilized, uninoculated, 4 = sterilized, inoculated with untreated soil of same type, 5 = sterilized, inoculated with untreated soil of another type, AH = early Hippophaë stage, HP = post-optimal Hippophaë stage

Graph 1.61 Variation in root length of Hippophaë seedlings with treatments of two soil types, AH and AP.



Key: as for Graph 1.60 above. (data from Oremus and Otten 1931)

The authors allude to further investigations in preparation concerning the adding of this nematode to young Hippophaë seedlings. In conclusion they felt able to state that Longidorus "plays a role in the degeneration of old natural stands of *H. rhamnoides*..." and "contributes to a reduction of the root system... and consequently reduced plant growth. The low nodulation seems to be the result rather than the cause of reduced root growth, but it contributes to it by affecting the nitrogen nutrition." (p.329)

Further experiments involved the addition of known numbers of Longidorus sp. and Tylenchorhynchus microphasmis to both sterilized and unsterilized dune soil from beneath a mature Hippophaë scrub. (MAAS, OREMUS and OTTEN 1983). The authors found a positive correlation between retardation of shrub growth and counts of the nematode species. A negative correlation was demonstrated between shoot nitrogen content, root nodulation, and the initial nematode count in a sterile soil. However some "unknown biotic factor" was also present reducing shrub growth, root nodulation, and also nematode multiplication.



#### 1.10 Seasonal variation in soil nitrate levels.

The cycle of growth, maturation and decay of Hippophaë stands has now been clearly established, and the work of Oremus and his associates has gone a long way to explain the underlying causes. Seasonal variation in soil nitrogen levels (i.e. during the course of a year) is a less-understood phenomenon in Hippophaë scrub, so that information on this process needs to be sought from studies of other types of habitat.

In a study of chalk grasslands (DAVY and TAYLOR 1974) the authors followed the seasonal variation in the release of inorganic nitrogen by the normal bacterial decomposition of soil humus. The dominant plant species was a grass - *Deschampsia caespitosa*. Although the decomposition of soil humus is probably not precisely comparable with the ageing and breakdown of the root nodules of Hippophaë, it is likely that both of these bacterial processes are influenced in much the same way by external climatic factors, and it is not unreasonable to anticipate parallels between them (*Deschampsia* is not recorded as a nitrogen-fixer).

Davy and Taylor collected soil samples on ten different occasions in the year from three different sites: an acid mull (pH 3.7-4.2), a calcinomorph brown earth (pH 7.2-7.8) and a chalk rubble (pH 7.6-8.3).

The soil samples were divided into lots and some were tested for nitrate - and ammonium-nitrogen directly, while other samples were incubated at a standard temperature of 25 °C or at the current soil temperature from which they had been taken.

The incubation at 25 °C was called "the potential mineralization rate" as it was the best that the soil bacteria could do, under ideal conditions. The incubation at current soil temperatures was termed "the actual mineralization rate" in that this was more likely to reflect what was occurring under field conditions.

The authors suggested that "seasonal changes in climate would be expected to have an important influence on, among other things, the size, composition, and activity of soil microbial populations, as well as the quantity and quality of organic matter available as substrate" (DAVY and TAYLOR 1974 p. 793).

In this examination of Davy and Taylor's paper attention has been concentrated primarily on the assays of soil nitrogen made at the time of sampling. These are most useful in indicating the natural seasonal variation in nitrogen, uncomplicated by artificial incubation in the laboratory. (Fig. 3 p. 799).

The authors commented that "generally concentrations (of nitrate - and ammonium-nitrogen) were low and of the same order of magnitude for the three soils. For most of the year the quantity of ammonium-nitrogen exceeded that of nitrate-nitrogen in all three soils, but the acidic soil fairly consistently contained the smallest quantities of nitrate-nitrogen, and the largest quantity of ammonium-nitrogen. Seasonal changes tend to run parallel in the three soils, especially so in the case of nitrate" (pp. 797-799).

Troughs of concentration were identified in April and late September, each followed by peaks in May and mid-November. In explanation of these peaks and troughs they suggested that "the spring peak in nitrogen mineralization may be attributed to the well known 'partial sterilization' effect of the winter climate, the death of a substantial proportion of the organisms in the soil having resulted in an abundance of protein substrate which was utilized when warmer conditions allowed the development of the appropriate microbial populations. The autumn peak might have been due to the input of organic nitrogenous substrate as litter, before the adverse winter conditions occurred." (pp. 803-4).

If the latter explanation of the autumn peak was correct it should have been possible to demonstrate the effect of extra litter not only as soluble nitrogen but also as an increase in total nitrogen. However, as "no increase in total nitrogen concentration in the soil was recorded" (p. 804) alternative explanations were sought. It was possible that "other factors may have affected the seasonal pattern e.g. soil water content... and the dryness may have contributed to the low rates of mineralization at this time" (p.104).

The incubation experiments revealed even bigger increases in the levels of soluble nitrogen with samples collected at the times of the peaks noted above. "Marked fluctuations" were recorded, and "the lowest potential mineralization rates were found in mid-winter December-January and to a lesser extent in April. Peaks were even more pronounced in April/May and to a lesser extent in November (p. 800).

The authors concluded by stating rather categorically that "the data for nitrate - and ammonium-nitrogen concentration in the soil at the time of sampling indicate what inadequate measures of soil nitrogen status these concentrations are, without the corresponding incubation data". The April trough probably indicates "that the rates of uptake by plants were exceeding the rapidly increasing mineralization rates at this time." The peaks in May indicate rapid mineralization in the increasingly warm weather.

This study is relevant to the soil chemistry of *Hippophaë* stands in that it is very likely that the release of nitrate-nitrogen from necrotic nodules of the roots is also influenced by the soil temperature, and in the winter months this process would slow down or cease. Temperature-sensitivity in bacterial groups is variable, and Davy and Taylor pointed out that "nitrifiers are less tolerant of sub-optimal temperatures than ammonifiers" but that "temperatures below 2 °C would be necessary to suppress nitrification sufficiently to cause ammonium to build up" (p. 803).

The explanations of April and November peaks in soil nitrate levels advanced by Davy and Taylor would only be applicable to mature stands of *Hippophaë*, as in the early stages the soil litter is virtually absent, and so is the soil microfauna. In established stands the surface layer of litter might appear large enough to account for peaks of nitrate accumulation, but the evidence suggests that litter in dune habitats makes an absolutely minimal contribution.

Increases in nitrate levels in dune soil would need to be

sought in terms of the activity of bacteria associated with necrotic nodules.

The data obtained by Davy and Taylor for rather thin chalk soils make an interesting comparison with the very poor dune soils. Originally they determined soil nitrate as ppm dry weight of soil. This was presented in Figs 2-6 as mgm per litre of dry soil using a "bulk density" value to convert from a mass to a volume. Fortunately the bulk density values were given in Table 1 p.796 so it is possible to convert the data to ppm simply by multiplying each one by the appropriate bulk density figure. These converted data are given in my Table 1.30 solely for the nitrate assays which were made at the time of sampling.

Table 1.30 Nitrate concentrations in chalk soil -  
seasonal variation. (Data from Davy & Taylor  
1974) -

Soil Type	Bulk g/ml Density	Collection times - monthly								
		J	M	A	M	J/J	A	S	N	D
Chalk	0.697 *	1.0	0.2	0.0	3.0	2.6	0.8	0.1	6.0	0.0
Brown earth	0.731	2.8	2.0	1.0	3.0	2.3	1.2	0.2	7.0	2.2
Acid mull	1.242	1.2	0.0	0.2	0.8	0.6	0.2	0.1	4.5	0.8
Chalk	- †	0.7	0.1	0.0	2.1	1.8	0.6	0.0	4.2	0.0
Brown earth	-	2.1	1.5	0.7	2.2	1.7	0.9	0.1	5.1	1.6
Acid mull	-	1.5	0.0	0.2	1.0	0.7	0.2	0.1	5.6	1.0

\* nitrate nitrogen in mgm/litre dry soil

† nitrate nitrogen in ppm dry soil

The values for nitrate are low, the largest being about 5 ppm for the organically rich mull but for most of the year the levels are below 1 ppm for the three soils. These compare closely with the results obtained in my dune soil study, indeed it is perhaps surprising that the levels were so low in the chalk soils derived from the felling of Chiltern beechwoods, where the general fertility might be expected to be higher.

1.11 Nitrogen fixation associated with marram grass -  
*Ammophila arenaria*

Within dune systems *Hippophaë* is the Nitrogen-fixer par excellence but there are other plant species with this propensity which occur in this habitat. The common marram grass, *Ammophila arenaria*, though not the earliest colonizer of dunes, is nevertheless the principal species responsible for effectively binding the shifting sands and creating the stability necessary for the later species to consolidate the habitat. It has been established in a series of studies that the success of *Ammophila* is partly due to the contribution of non-symbiotic bacteria like *Azobacter* which are found in close proximity to the rhizosphere of the plant, but which have not developed a close cytological and physiological union with the tissues (HASSOUNA 1962; HASSOUNA and WAREING 1964, ABDEL WAHAB 1975, and AHMAD and NECKELMANN 1978). More than one bacterial species seems to be involved and several strains of *Bacillus* were identified as N-fixers by the acetylene reduction assay and proposed as new species on the basis of their physiological potential (ABDEL WAHAB 1975).

The rhizosphere of *Ammophila* is extremely extensive, going down to depths of six metres or more, and spreading laterally by means of rhizomes. It was always assumed that this large rhizosphere was concerned with the problem of obtaining sufficient water in a rapidly draining sandy soil. However, it is only the top 10 cm of dune sand which is completely dry, and even in hot, dry, summers the sand at a depth of one metre is always cool and damp.

It is possible that the function of the extensive rhizosphere is to provide a big surface area for the absorption of the products of N-fixation by bacterial colonies in the soil.

Although the dune soil bacteria around *Ammophila* have been described as "non-symbiotic" by the workers cited earlier, and there is no evidence of a cytological union between the two species, it seems clear that a loose physiological association does occur, and this might well be termed symbiosis. It has been shown that when the bacterial colonies are cultured they grow better and fix more nitrogen when carbohydrates like glucose are present (ABDEL WAHAB 1975). The presence of mannitol was shown to aid growth also (ABDEL WAHAB and WAREING 1980).

The importance of root exudates by *Ammophila* has been stressed by Abdel Wahab and Wareing and these probably contain simple carbohydrates, which would enhance the growth of nearby bacteria. Indeed it appears that these bacteria exist in very low numbers away from the rhizosphere of *Ammophila*, and only occur in large numbers on the root surfaces and rhizosphere sand. (HASSOUNA 1962, HASSOUNA and WAREING 1964, ABDEL WAHAB 1969).

Hence there is a two-way benefit, and at least the makings of a symbiotic relationship. The plant can readily synthesize sugars as it only needs carbon dioxide and water as substrates. A fraction of these can be passed on to the bacteria as root exudates. As there is no soil litter to break down, the bacteria will be seriously limited in their growth by a lack of carbohydrates, so the exudate will form a crucial part of the basic food intake. The lack of litter,



with nitrogen, constitutes a major limiting factor for the plant, so the nitrogen fixed and passed on by the bacteria constitutes an essential part of the food intake. Such a mutual dependence could have arisen easily as any *Ammophila* strains capable of a tendency to exude at the roots would stand a much better chance of surviving in the hostile environment of bare sand dunes.

This situation has been described as 'associative symbiosis' (ABDEL WAHAB and WAREING 1980) largely because of the difficulty in "assessing the extent to which this... contributes to the nitrogen nutrition of the plant under natural conditions" (p. 718). However, under natural conditions where else would *Ammophila* get its nitrogen? By the time that an effective leaf litter surface had developed *Ammophila* has faded away unable to compete with other plant species. It only flourishes where the sand is loose and the litter is almost non-existent.

It is true that some nitrogen will be supplied in rain and sea-spray but it is not unreasonable to suspect that N-fixation may provide the major bulk. Despite the fact that the small experimental plants used by Abdel Wahab and Wareing had no opportunity to develop the extensive rhizosphere systems exhibited by plants under natural conditions the results of nitrogen accumulation have been impressive.

My interest in this study of *Ammophila* lies in the question of how much the 'associative symbiosis' affects dune soil fertility and increases levels of nitrogen. A closer examination of the results of Abdel Wahab and Wareing 1980, now follows.

The authors principally measured total nitrogen or rates of nitrogen-fixation under different conditions, and did not estimate solubles like nitrate- or ammonium-nitrogen in soil. In an initial experiment to obtain an overall "nitrogen balance sheet" they grew seedling *Ammophila* plants in pots of dune soil over a period of two years, and ten months. A series of controls were used which contained no *Ammophila* plants. Their data (Table 1 p. 715) have been re-arranged and expanded as my Table 1.31 below. The total nitrogen content of the dune soil and the plants was assayed at the start and again at the end of the experimental period to enable the gain of nitrogen to be calculated.

Table 1.31 Increase in total nitrogen due to bacterial N-fixation. (Data from Abdel Wahab and Wareing 1960)

Treatment	START		FINISH		INCREASE	
	C	Ex	C	Ex	C	Ex
Sand	132.60	132.60	147.520	317.805	14.920	185.205
Plant	-	.082	-	32.466	-	32.384
Total	132.60	132.682	147.520	350.271	14.920	217.589

Data in cells are total Nitrogen in mg

C - control (without plants)      Ex - experimental (with plants)

Sand - sand surrounding plant, or sand alone

Plant - nitrogen of plant, including dead leaves etc.

The results reveal that left to itself, dune soil with its bacterial flora can increase the nitrogen content. Over the near three-year period the level of nitrogen went up by

14.92 mg. This is an increase of about 11% on the initial concentration. It is a modest but significant change. The level of nitrogen initially is given as "average amounts" for 20 pots, and each contained 3 Kg of sand. The figure of 132.6 mg nitrogen therefore represents 44 ppm which is surprisingly high.

When a marram plant is present in a pot the overall increase in nitrogen is much more dramatic, and 217 mg represents an increase of 163%. Presumably this rise is accounted for by the relationship between the plant and the bacteria, as neither by themselves could achieve this result.

It is interesting that the nitrogen formed is not concentrated in the plant, but most of it is in the soil. The gain in soil nitrogen was 185 mg while the plant gain was only 32 mg. Thus the shift in proportion of soil N: plant N from the start (virtually 100% : 0%) to the finish (86% : 14%) was quite modest.

The authors were unsure as to the form in which the nitrogen occurred in the sand "but some of it must have represented dead remains of roots... thus a significant part of the carbon source for the fixed nitrogen may have come from the dead or decorticated roots rather than from root exudates" (p. 714).

It is important to note that the symbiotic relationship need not be quite as close as the simple exchange between exudate and fixed-nitrogen suggested earlier. It remains true that the only source of carbohydrate available to the bacteria is that derived from the marram plant. If it is in the form of

dead roots it represents a symbiosis at one remove from that indicated by the "exudate hypothesis".

It would have been interesting to know what proportion of the soil nitrogen was in the form of dead roots. If it was in some other form it could provide additional weight to the idea of the importance of exudates to bacterial nutrition.

The authors went on to examine by acetylene reduction assay the amount of N-fixation achieved by the bacteria/marram root association. Surprisingly in every case it was much lower than the levels of total nitrogen would have led one to expect. "In both experiments the observed rate of nitrogen-fixation... was very much lower than the average gain in nitrogen by the plants as determined by the Kjeldahl method" (p. 715).

To have accumulated, over nearly three years, the amount of total nitrogen measured it would have been necessary for the bacteria/plant association to fix at the rate of about 32  $\mu\text{g}$  nitrogen per g of root per day. The potted plants only fixed about 1  $\mu\text{g}$  per g per day. As those plants "were in a rather moribund condition" (p. 714) the authors tried healthy younger plants less than one year old but these only fixed 2  $\mu\text{g}$  per g per day.

Then fresh samples were assayed from marram growing under natural conditions, but these only fixed about 1  $\mu\text{g}$  per g per day. Even when the rate of fixation of the plants in situ within the dunes was measured it was no more than 2.2  $\mu\text{g}$  per g per day.

How was it possible for the plant/bacteria association to accumulate so much nitrogen at such low fixation rates? The answer seems to lie in the adequate nutrition of the bacteria. When a soluble carbohydrate substrate was added to the assay samples the fixation rates increased greatly. A solution of 0.5% glucose increased fixation by six times under aerobic conditions, and up to sixteen times in anaerobic conditions (p. 715).

As the authors commented, "lack of a carbon source or energy supply was limiting their (the bacteria's) nitrogen-fixing activity" (p. 715). They went on to carry out a further series of experiments in which nutrients containing substances like biotin, potassium ions, phosphate ions, and mannitol were added to marram root samples prior to assay for N-fixation. However "the average daily rate of gain in total nitrogen in the long-term pot experiment greatly exceeded the highest rate of N-fixation measured by the acetylene reduction method." (p.717).

This remained the major question in this series of experiments, and the authors discussed some possible reasons for the discrepancy. They noted that rates of fixation above those measured would "depend upon the amount and nature of the root exudates... and this is likely to be markedly affected by such environmental factors as temperature, water supply, and light intensity." (p. 717).

There are also "seasonal variations in the rate of acetylene reduction in samples taken from the field, with a maximum occurring in June." (p. 719). Higher fixations would be expected "when the sand is saturated after rain (and) the

roots will be under relatively anaerobic conditions."

(p. 719).

Errors due to an imperfect conversion factor between acetylene reduction and nitrogen-fixation were discussed, and although the need to determine this conversion factor under the experimental conditions used was recorded, it was not possible for the authors to resolve the difficulty in this particular study.

Even these considerations did not seem sufficient to account for the large discrepancy revealed by the experiments. It was felt "more likely that the nitrogen gain in the sand arose from the activities of micro-organisms using dead root residues as a carbon source rather than exudates from living roots" (p. 719).

This dilemma could only be resolved by a programme of research designed specifically to examine the problem of exudates versus dead root tissue in bacterial nutrition. What, for example, is the chemical nature of the marram root exudates? It is possible that the plant has evolved a secretion which is ideally tailored to the needs of bacteria to promote maximum N-fixation? If dead root tissue makes the greater contribution, it should be possible to demonstrate this by artificially increasing the level of dead roots to see if the rate of fixation increases correspondingly.

This particular study has revealed further fascinating problems in the nitrogen chemistry of dune soils, and an interesting parallel with *Hippophaë* in that both this and marram can only exist successfully in the poorest dune soils, and are killed off by competition, and both are associated with nitrogen-fixing micro-organisms.

Chapter Two:    Experimental design of the project -  
                 a seasonal study of nitrate levels in dune  
                 soils associated with sea-buckthorn.

## 2.1 Some initial questions

Dune soils have been studied for many years, in parallel with examination of the dune succession in particular localities. The study of nitrogen in dune soils had been somewhat neglected, until the advent of more modern analytical techniques, because nitrogen levels are generally low, and difficult to measure accurately. Dune sand is perhaps an ideal proto-soil to analyse in that it is initially little more than an inert matrix of quartz particles, with few solubles of nutritional value to plants. Yet within a space of thirty years such a soil may change sufficiently to support a rich and varied flora and fauna. These changes largely result from the entry of new materials to the soil rather than breakdown of material already present in the mineral matrix.

### 2.1.1 Scrub maturity

Some of the earliest colonizers of bare sand (*Ammophila arenaria*, *Hippophaë rhamnoides*) are known to fix atmospheric dinitrogen. Do they make a significant contribution to increasing dune soil nitrogen levels? These levels, low at first, might be expected to increase with the degree of successional maturity of buckthorn scrub.

### 2.1.2 Effect of litter

Will colonization itself increase the levels of soil nitrogen, due to the death and decay of plant and animal matter within the surface litter? It is necessary to try to discriminate between the enhancement of soil nitrogen as a



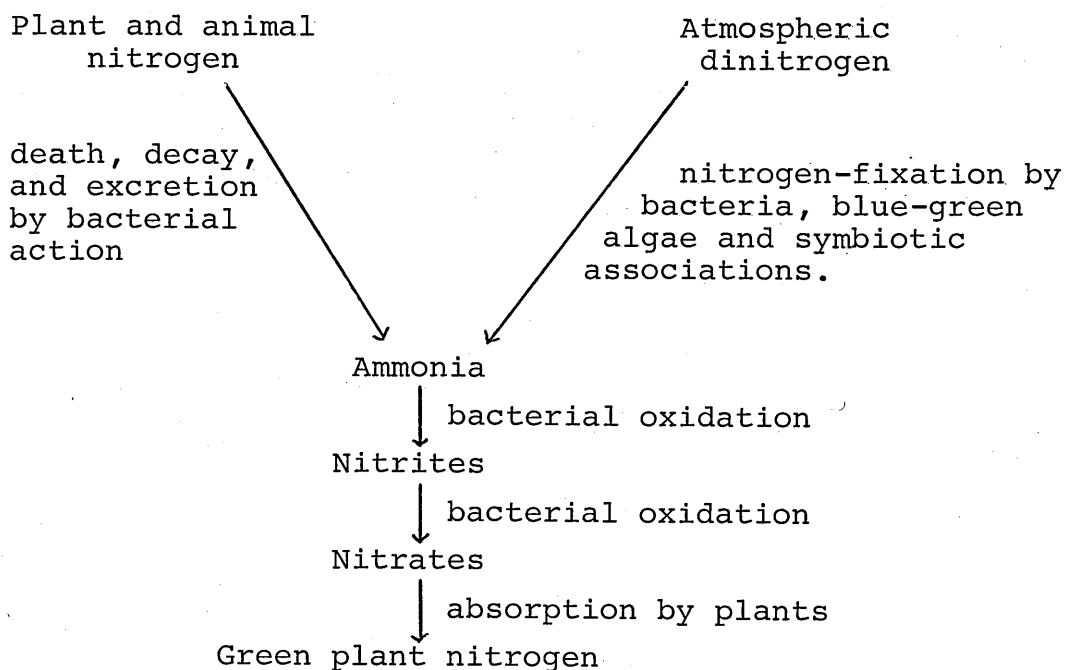
result of litter decay, and as a result of nitrogen-fixing activity by colonizing plants. (Fig. 2.1)

It is possible to prevent the downward percolation of nitrogenous material from the litter by an impervious barrier. The contrast in nitrogen levels between "covered" experimental sites, and untreated control sites should demonstrate whether the main contribution is made by litter or nitrogen-fixation.

### 2.1.3 Type of scrub

What is the value of nitrogen levels in a dune scrub lacking nitrogen-fixing colonizers, for example that dominated by *Crataegus*? If soil nitrogen levels in a hawthorn scrub are much like those in a buckthorn scrub then one might conclude that leaf litter or the death and excretion of animals provide the main source of nitrogen. If, however, there is a distinct superiority in the nitrogen-status of the soils of buckthorn scrub, then it seems likely that the effects of the decay of surface litter are only marginal and explanations may be sought in the nitrogen-fixing potential of the root nodules of the sea-buckthorn.

Fig. 2.1 Routes of nitrogen in dune soils



#### 2.1.4 Animal Excretions

An indirect method was used to provide an additional test to discriminate between nitrogen derived from the break-up of nitrogen-fixing nodules in the soil, and nitrogen percolating downwards from the excretory products of animals. This was the analysis of soil samples for the presence of soluble phosphate.

Mammal and bird droppings contain high levels of soluble phosphate. If increasing levels of nitrogen with increasing maturity of scrub were also associated with increasing levels of phosphate it would suggest that animal excretions were responsible. Mature buckthorn provides cover for rabbits, nest sites for birds, and orange berries as food for birds. Immature buckthorn is far less attractive to animals. A fairly constant level of phosphate would indicate a background derived from the slow solution of fragments of sea-

shells in the sand.

Similarly, if nitrogenous excretions were prevented from percolating downwards at the experimental sites, phosphate would also be prevented. A difference between the levels of phosphate at control sites and at experimental sites would suggest that the excretory activities of animals were a significant factor in contributing to phosphate, and by association, nitrogen levels.

#### 2.1.5 Seasonal variation

There are some grounds for expecting the levels of soil nitrogen to change during the course of the year (DAVY and Taylor 1974). This is generally associated with changes in soil temperature, and the activity of bacteria, in metabolizing nitrogen compounds more rapidly at higher temperatures.

Although there will be a wide range of reactions going on at any one time, and the measurement of one or two ions will provide only the most general information it is still useful to gain some idea of the kinds of seasonal fluctuations occurring in dune systems. Is there an upturn in nitrogen levels during the course of the year?

#### 2.1.6 Air pollution

Atmospheric nitrogen compounds derived from industrial pollution can enter dune soils in rainfall. It has been suggested that this may contribute 1.5-2.5% of the annual input of nitrogen to the system (BOORMAN 1977 p.171). The value depends on prevailing winds and the proximity of urban areas. At maximum the effect of this would be marginal on values of soluble

## 2.2 Experimental design

The study was conceived as one main project with a number of subsidiary ones, together with various checks and controls. The variables include: the level of soil nitrate, the season of the year, the effect of litter solubles, the age of the sea-buckthorn scrub, the type of scrub, and the nature of the dune locality. The main project was limited to the first four variables to allow for some replication of results while reducing the problem of handling and analysing a large number of samples. The last two variables were partially investigated in subsidiary projects.

### 2.2.1 Soil nitrate

This was the main numerical variable, expressed as parts per million dry soil or millionths of a gram of nitrogen as nitrate per gram of dried dune soil. It was this data which forms the basis of all the graphical and statistical analysis.

### 2.2.2 Season of the year

Soil samples were collected on seven occasions during 1981, namely February, April, June, August, September, October, and November. The soil nitrate levels were expected to show a spring increase (with warmer weather) followed by a return to a relatively constant level for the rest of the year. This variable could be presented graphically by plotting the mean nitrate level, with standard error of deviation against the season of the year. If the data warranted this it would be possible to carry out significance testing.

### 2.2.3 Effect of the litter solubles

Control sites were left unaltered, so that litter solubles could percolate down in the soil, while at experimental sites percolation was interrupted by sub-surface polythene sheets. The null hypothesis here, is that there is no significant difference between levels of soil nitrate in control or experimental sites within a particular age group of scrub. This can be tested statistically by the 'Student's' t test of significance between treatments (control or experimental). When the variance ratio F has been found for each case (the three scrub ages) it can be compared with the table values of F at the usual 1% or 5% levels of significance.

### 2.2.4 Age of sea buckthorn scrub.

Three age-groups were selected; young (2-3 years - mean 2.5 years), medium (4-5 years - mean 4.5 years) and old (11-14 years - mean 12.5 years). The data could be presented graphically by plotting the mean soil nitrate levels, with standard error, against scrub age for control and experimental sites. Significance testing was appropriate if the standard error was too great in a graphical presentation. The expectation was that nitrate levels should be higher under older scrub.

### 2.2.5 Type of scrub

If soil nitrate beneath scrub is due to percolation of litter it ought to be possible to show this in non-Hippophaë dune scrub. The subsidiary projects examined such scrub sites.

The data could be presented graphically or analysed statistically.

2.2.6 Nature of dune locality

The study encompasses both "acid" dune systems in the west and the more alkaline dunes of the east coasts. The comparisons can be presented graphically or statistically. It was anticipated that the nature of the scrub would be conditional upon the dune locality.

Thirty five soil samples were normally collected for analysis on most occasions as indicated in the table below.

	Age of Scrub						
	B	Young		Medium		Old	
	C	C	E	C	E	C	E
Five replicates							

KEY

B = sea beach

C = control site

E = experimental site

The data in the cells are concentrations of nitrate in parts per million dry soil.

## 2.3 Principal project - experimental details

### Hunstanton - Holme, Norfolk

A wide range of checks and controls on methods and accuracy were used. The following account has been streamlined by omitting any discussion of problems, or inherent variation encountered. A full examination of these aspects is given in Chapter 3 "Sources of error".

#### 2.3.1 Nature of the area (O.S. Grid Reference TF690440)

The main longitudinal study of the seasonal variation in dune soil nitrogen levels was carried out in the Hunstanton-Holme area of N.W. Norfolk. (Map 2.1) This area of dunes and slacks is owned in part by the Hunstanton Golf Club, and in part by the LeStrange Estates. Both organizations readily gave permission for the experimental procedures carried out within the dune system. Although the area is privately owned the general public have easy access from the beach to the dunes, or via the car park at Holme, and no attempt is made to keep them out, as long as they keep off the greens of the Golf Course.

There is no record of sea-buckthorn being planted as management policy either by LeStrange Estates or the Golf Club, and it is assumed that the plant has propagated naturally from seeds in the droppings of birds, or has been deliberately introduced by persons unknown. The nearest sizeable buckthorn scrub is 2 Km to the east on dunes owned partly by the Holme Nature Reserve, and partly by the Holme Bird Observatory. (Map 2.2) The extensive buckthorn thickets at Gibraltar Point, Lincolnshire are only 20 Km away

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from the study site in a direct line of flight across the waters of the Wash (Map 2.1).

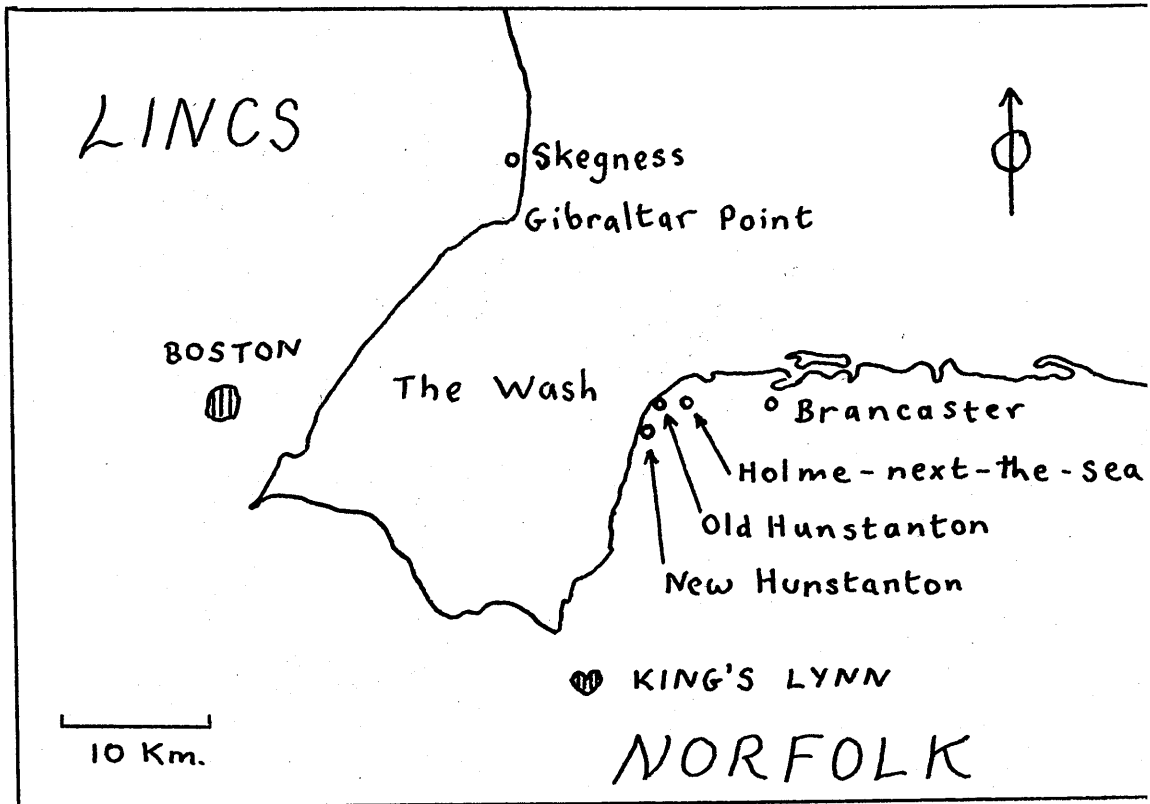
The whole question of the nature of sea-buckthorn as a native or introduced species is a debatable one. There is evidence that it was widely distributed after the end of the last glaciation in northern Europe, but it retreated with the spread of the broad-leaved forest. In western Europe the coastal populations extend northwards from the north French coast, and the inland populations occur on most mountain ranges in continental Europe. In both cases the soils are very poor, rich in calcium, and free of competition from other species, as buckthorn is very intolerant of shade conditions (PEARSON & ROGERS 1962).

The sea-buckthorn scrub at Hunstanton - Holme is indicated as native (GROVES 1958) and recorded as such in the current "Atlas of the British Flora". The Biological Records Centre, Monks Wood, Huntingdon have a record of sea-buckthorn in this area for 1933, identified by C.E. Hubbard. However the precise status of the sea-buckthorn colony does not affect the study in a material way.

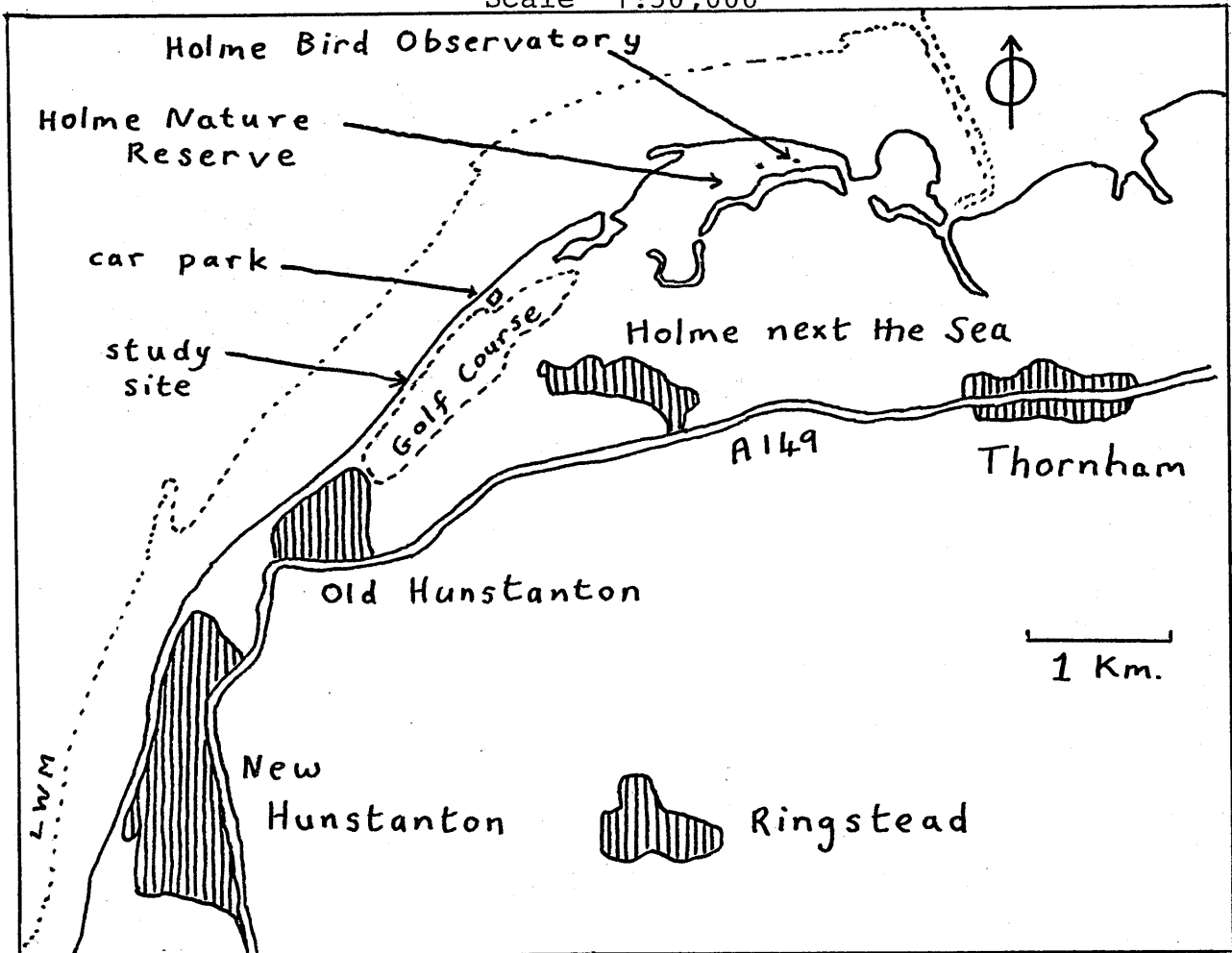
The dune area at Hunstanton-Holme consists of a long bare ridge above the beach (Map 3) colonized by immature buckthorn plants less than 0.5 m high. This ridge is rather poorly colonized by marram, perhaps because it is used as a road by tractors owned by the Golf Club. The ridge top is flattened most distinctly, in contrast to the contiguous ridge to the east of the Holme Car Park. Here the ridge top is steep and undulating, with an excellent growth of marram grass. It is known that foot-trampling has an adverse effect

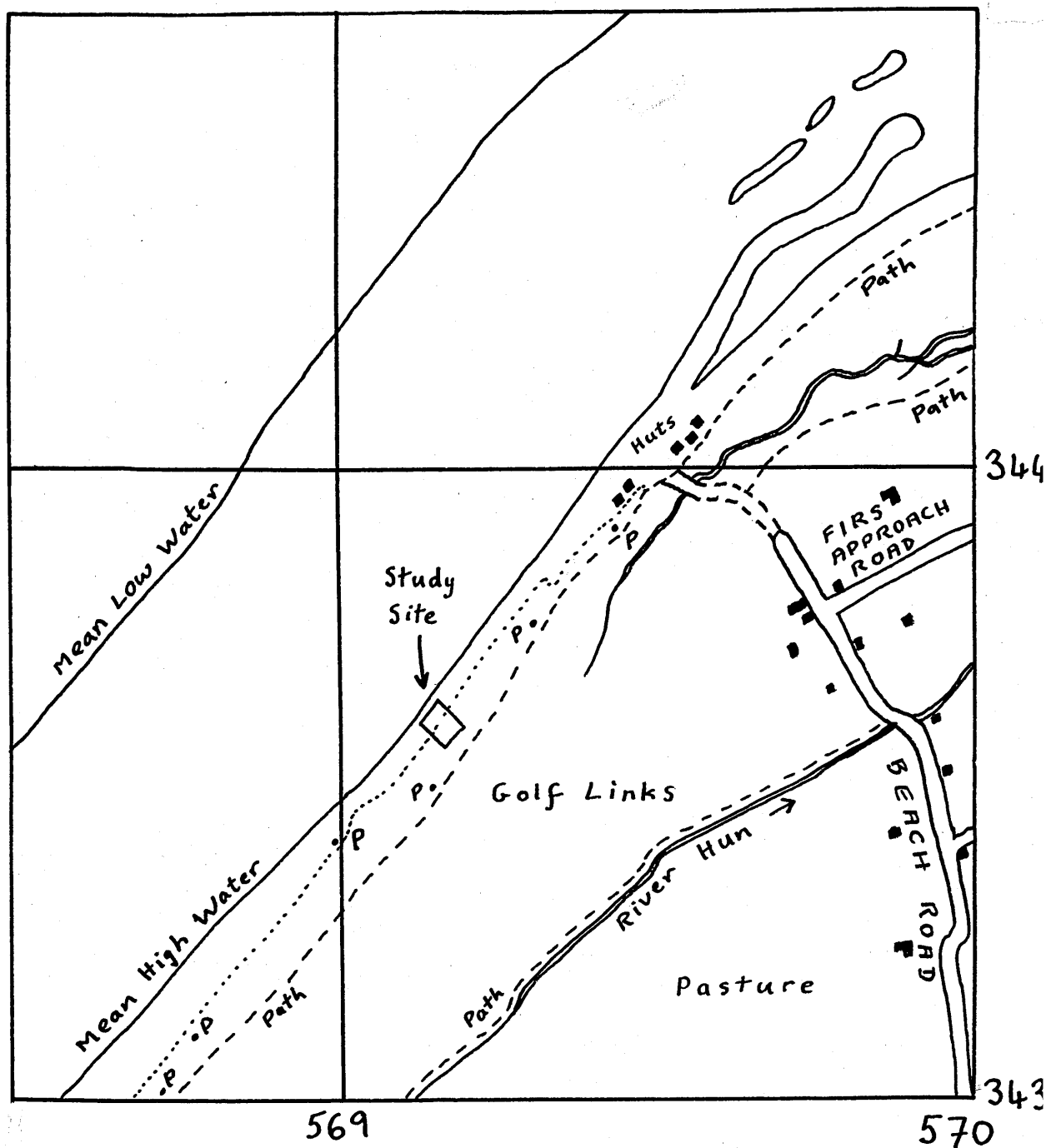


Map 2.1 North-west Norfolk  
Scale 1:500,000



Map 2.2 Holme-Hunstanton area  
Scale 1:50,000



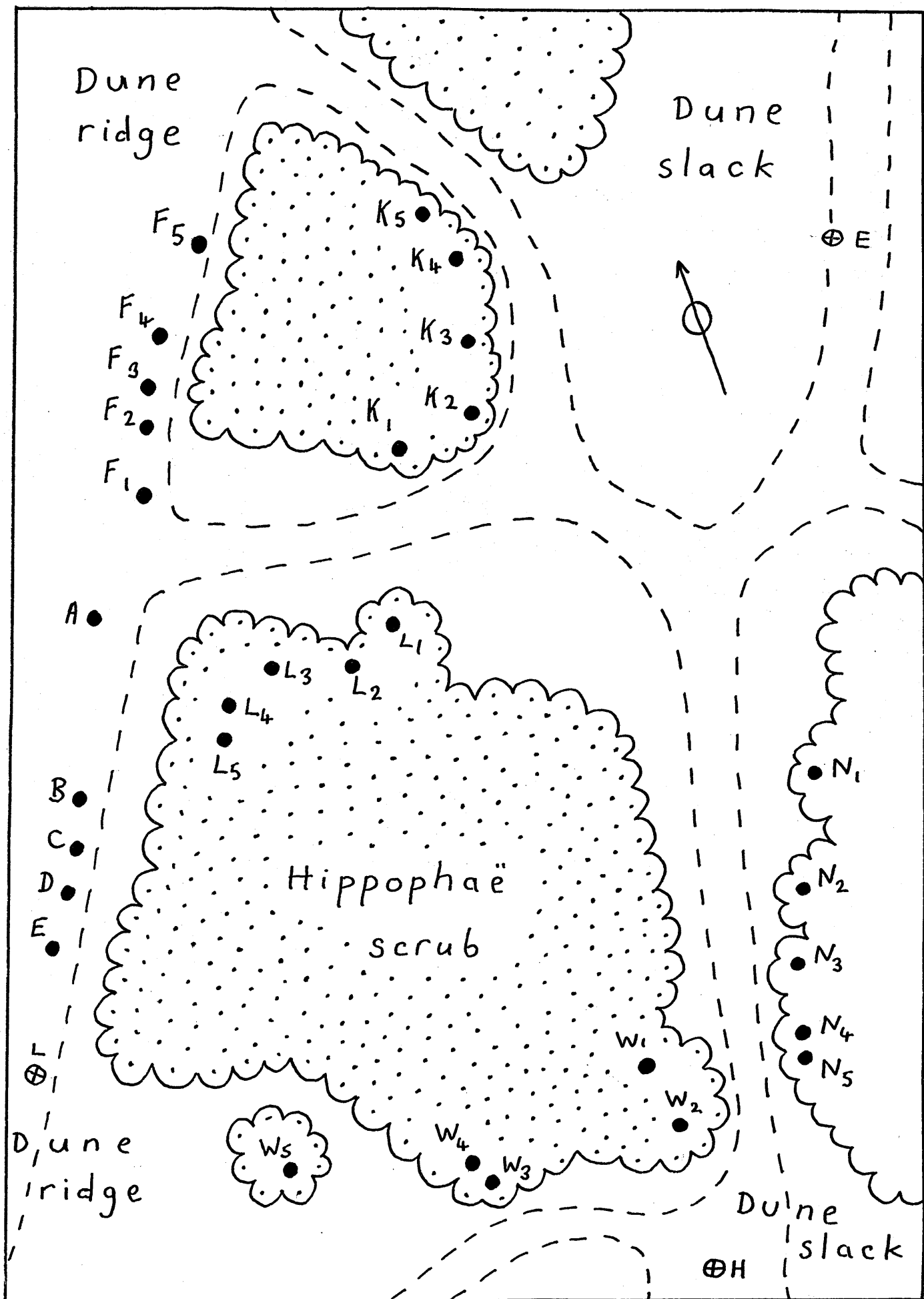


Key .P marker Posts  
 ■ wooden beach huts

0 100 m 1000 m

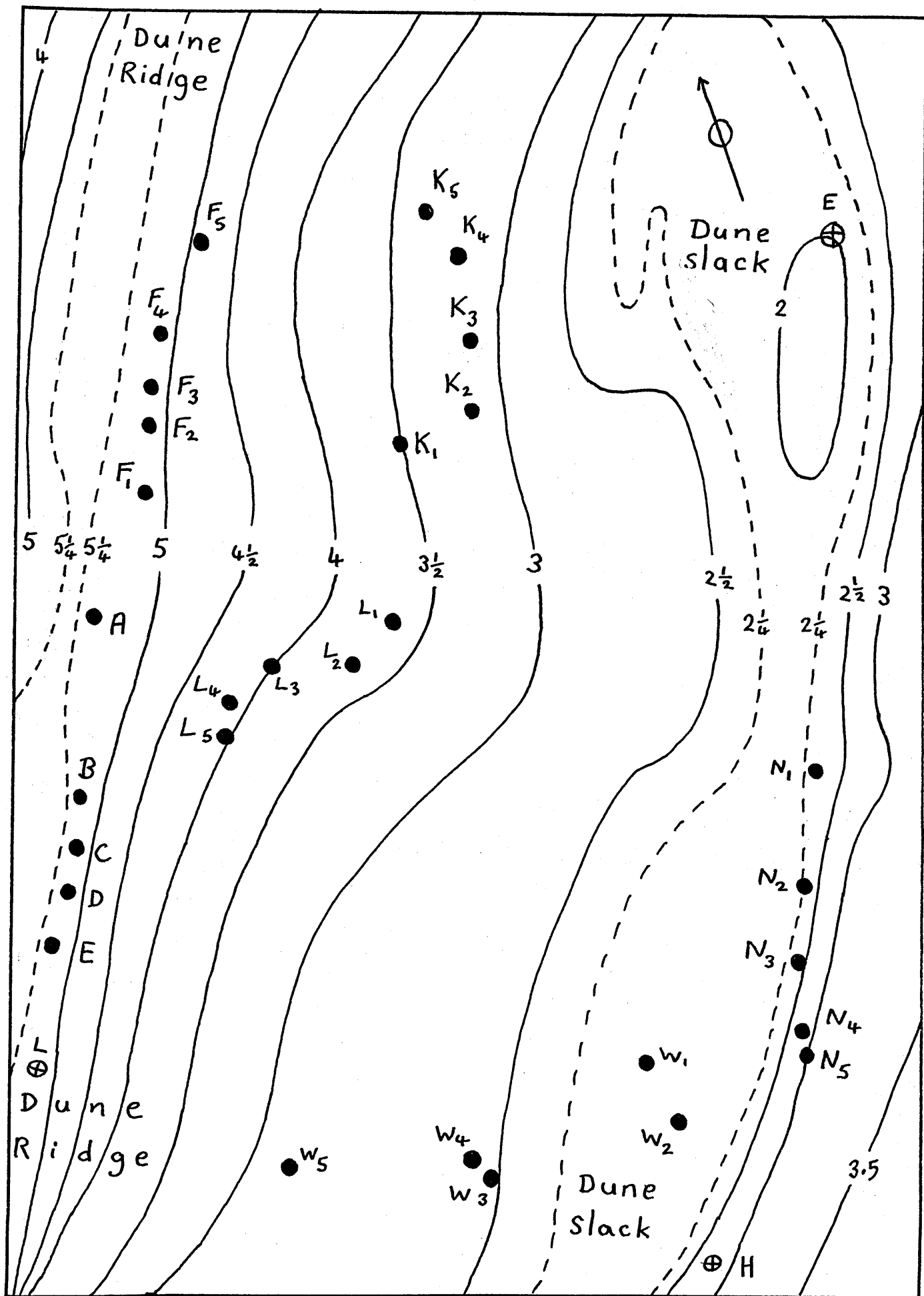
Map 2.4 A Principal study area: Hunstanton-Holme. Map of Hippophaë scrub and soil sample sites (A-W5)

Key: ⊕ E,L,H survey points



Principal study area: Hunstanton-Holme. Contour map and soil sample sites. Height in metres above arbitrary datum.

⊕ E,L,H triangulation and levelling points



upon marram (BOORMAN & FULLER 1977, TREW 1973) and the frequent passage of a caterpillar tractor is probably sufficient to prevent marram developing altogether. However the buckthorn seems to be able to survive this treatment.

Between this bare ridge and the next one is a damp dune slack with a range of buckthorn bushes, but primarily an open scrub of individuals up to 1 m high, and occasional clumps of dense scrub up to 2 m high (Map 2.3). The inner ridge has a continuous line of dense scrub running along the seaward face for several hundred metres. Further inland from the inner ridge, the pattern of dune and slack has been disturbed by the creation of a golf course (Map 2.3) but large isolated clumps of mature buckthorn, over 3 m high, stand out among the greens.

The largest scale of map available was the 6" to 1 mile Ordnance Survey, so it was necessary to make a plane-table survey of the area, to determine the dimensions of the scrub thickets in the region in which the soil samples were taken. A number of sections were made with a level, to record the small-scale rise and fall of the terrain, at the study site. Map 2.4 was prepared from the results of the levelling, and plane-table survey.

### 2.3.2 Sea-Buckthorn scrub sites

The individual bushes, chosen as a sample of the general population, were divided into three groups on the basis of their approximate general size, in terms of the height, stem diameter, and extent of growth. Data are available on these

parameters in section 3.4. Each of the three age-groups consisted of ten bush sites; five controls and five experimental sites.

At each experimental site, a sheet of thick polythene (area  $1 \text{ m}^2$ ) was laid around the buckthorn bush, 10 cm below normal soil level. Once the sand was replaced the sheet was secure against lifting by the wind, and was hidden from casual observers. The control sites were left untreated. Each bush was tagged, low down, with an anodised aluminium label. The youngest bushes on the bare ridge were also marked with a section of coarse nylon net. This was necessary because the bushes were covered by increasing layers of sand, and the net was readily visible but not so unusual as to excite the interest of the casual observer.

Beach sand samples were also collected as part of the controls procedure, from a region below the bare ridge crest, and the extreme high water of the spring tides.

### 2.3.3 Methods of soil sampling

Soil samples were collected at intervals throughout the year, from December 1980 to November 1981. The earliest results were in the nature of a pilot study, to see if there were measurable levels of nitrogen-bearing ions in dune soils, and to standardize the analytical technique.

The main method of collecting soil samples was by means of a soil auger, diameter 35 mm, driven to a depth of 0.4 m before extraction. This depth was chosen on the basis of nodulation studies. (OREMUS 1979, VASCHENKO 1973). Each

sample was taken close to the centre of bush, and consisted of the material held by the terminal 20 cm of the flutes of the auger, amounting to about 150-200 g. This was placed in a new polythene bag, labelled, and secured with a rubber band.

All samples were refrigerated on the evening of the collection day, at a temperature of 8 °C. In most cases the extraction procedure commenced the next day, so that the likelihood of bacterial action on the nitrogenous material present was minimised.

Table 2.1 Sample references collections and determinations

Sample Numbers	Collection Date	Determination Date	Location	Notes
001-015	19.12.80	12.1.81	Hunstanton	Pilot study range of tests
019-035	29.12.80	10.1.81-3.3.81	Hunstanton	Surface and depth
040-069	13.2.81	10.3.81-26.3.81	Hunstanton	S1
070-099	6.4.81	20.5.81	Hunstanton	S2
DV1-DV11	22.4.81	1.5.81	Braunton, Devon	Scrub/beach comparison
C1-C20	25.5.81	5.6.81	Daymer, Cornwall	Scrub/beach
R11-R43 S11-S55	27.5.81	5.6.81	Studland, Dorset	Scrub/beach
100-134 N1-N6	3.6.81	12.6.81	Hunstanton	S3 depth study
140-154	14.7.81	17.7.81	Gibraltar Point, Lincs	Scrub/beach
155-189	25.8.81	28.8.81	Hunstanton	S4
191-225	23.9.81	29.9.81	Hunstanton	S5
226-260	14.10.81	29.10.81	Hunstanton	S6
261-295	4.11.81	9.11.81	Hunstanton	S7

S - standard sample collections from marked sites

#### 2.3.4 Extraction procedure

Each soil sample was sieved through a 4 mm mesh to remove gravel, and larger particles of organic material and then divided into two lots, a major bulk, and a minor bulk, about 120 g and 60 g respectively. The minor samples were weighed,



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dried at 80 °C for 24 hours, and re-weighed to determine the water content. In some cases these samples were kept for further analysis.

Each major bulk sample was weighed and irrigated with about 50 ml of de-ionised water for 2 hours at room temperature. The lixivate was filtered through glass wool to remove sand and organic fragments, and filtered through 10 grade "Postlip" papers. When haze prove troublesome the filtrate was passed through a suspension of calcium carbonate in a sintered Büchner funnel. The major bulk samples were normally discarded after extraction, but in some cases further analysis was made.

Extraction was performed on the wet sample (major bulk) because this reduces by one day, the delay before analysis. In addition samples in the drying oven would still contain nitrate but a proportion of ammonium ions would be lost to the atmosphere as ammonia gas.

Concentrations of ions were expressed as ppm dry weight of soil. Having used a wet sample for extraction it is necessary to convert this to the "corresponding dry weight". This can be done by multiplying the wet weight by a conversion factor, R, for each sample, derived from measurements on the minor bulk sample.

$M_w$  = minor bulk, wet weight

$M_d$  = minor bulk, dry weight

$W_w$  = major bulk, wet weight

$W_d$  = corresponding dry weight

$$\frac{M_d}{M_w} = R$$

$$W_d = W_w R$$

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The quantity of soluble extract from each soil sample was noted; this varied from one sample to another depending upon difficulties of extraction. It proved more convenient to simply note the mass of soluble extract S, rather than attempt to standardize the precise amount of liquid used.

Extracted solubles, in water were subject to analysis for nitrate or ammonia on the following day. Tests for chloride or phosphate were done later because the likelihood of bacterially induced change was rather less.

#### 2.3.5 Analysis for nitrate ion

The procedure followed for the analysis of nitrate ion is based the nitration of phenoldisulphonic acid. (PDSA) Dry nitrate will react with PDSA in concentrated sulphuric acid to produce a compound which turns a vivid yellow in a strongly alkaline solution. The intensity of the yellow colour is proportional to the amount of nitrate added to the PDSA solution.

The various modifications of the technique, and necessary precautions are discussed fully in section 3.4 "Sources of error"; the following is the standard method.

A series of 10 ml aliquots from the known masses of each soluble extract were pipetted into labelled 40 ml vials. Each of these solutions were evaporated to dryness with anti-bumping granules. Along with the test solutions, a series of 10 ml samples of ammonium nitrate solutions of known strength were also evaporated as standards to calibrate the spectrophotometer.

On cooling, each vial was charged with 1 ml of a solution of 25% phenol-disulphonic acid w/v in concentrated sulphuric acid, and the reaction was allowed to proceed for fifteen minutes. The acid solution was diluted with about 10 ml of de-ionised water and allowed to cool. Then about 10 ml of nitrate-free .880 ammonia solution was added to each vial to neutralize the acid, and develop the yellow colour. On cooling each sample was made up 30 ml with ammonia, and the vials were stoppered.

The test solutions, and standard solutions were examined in the spectrophotometer on the following day but the coloured solution is stable enough to permit a delay of several days.

The absorbence of these solutions was measured at a 420 nm wavelength. A pair of matched, 1 cm path-length quartz cuvettes was used. The reference standard was a solution of 1 ml PDSA in 30 ml .880 ammonia. Initially the absorbence of the reference standards was measured to ensure that a linear relationship between nitrate concentration ( $\mu\text{M}$ -micromolarity) and absorbence was demonstrable.

The test solutions were then placed in turn in the cuvette and the absorbencies were recorded.

The nitrate concentrations were calculated as nitrogen as nitrate in parts per million of dry soil.

([N] as  $\text{NO}_3^-$  ppm dry soil)

x = concentration of standard nitrate solution ( $\mu\text{M}$ )

y = absorbence of standard nitrate solution

Conversion factor,  $C = \frac{X}{Y}$

$K$  = concentration of test nitrate solution ( $\mu\text{M}$ )

$A$  = absorbance of test nitrate solution

The 10 ml aliquots of the standards, and the soluble extracts were treated identically, so as  $x = Cy$  then  $K = CA$  and  $K$  = concentration of nitrate in soluble extract.

1,000 ml 1M ammonium nitrate contains 14 g nitrogen as  
nitrate

$\therefore$  1,000 ml  $K\mu\text{M}$  ammonium nitrate contains 14  $\mu\text{g}$  nitrogen as  
nitrate

$S$  ml  $K\mu\text{M}$  ammonium nitrate contains  $\frac{14 KS}{10^3}$   $\mu\text{g}$  nitrogen as  
nitrate

$W_d$  g dry soil contains  $\frac{14 KS}{10^3}$   $\mu\text{g}$  nitrogen as nitrate

( $S$  ml of soluble extract prepared from  $W_d$  g dry soil)

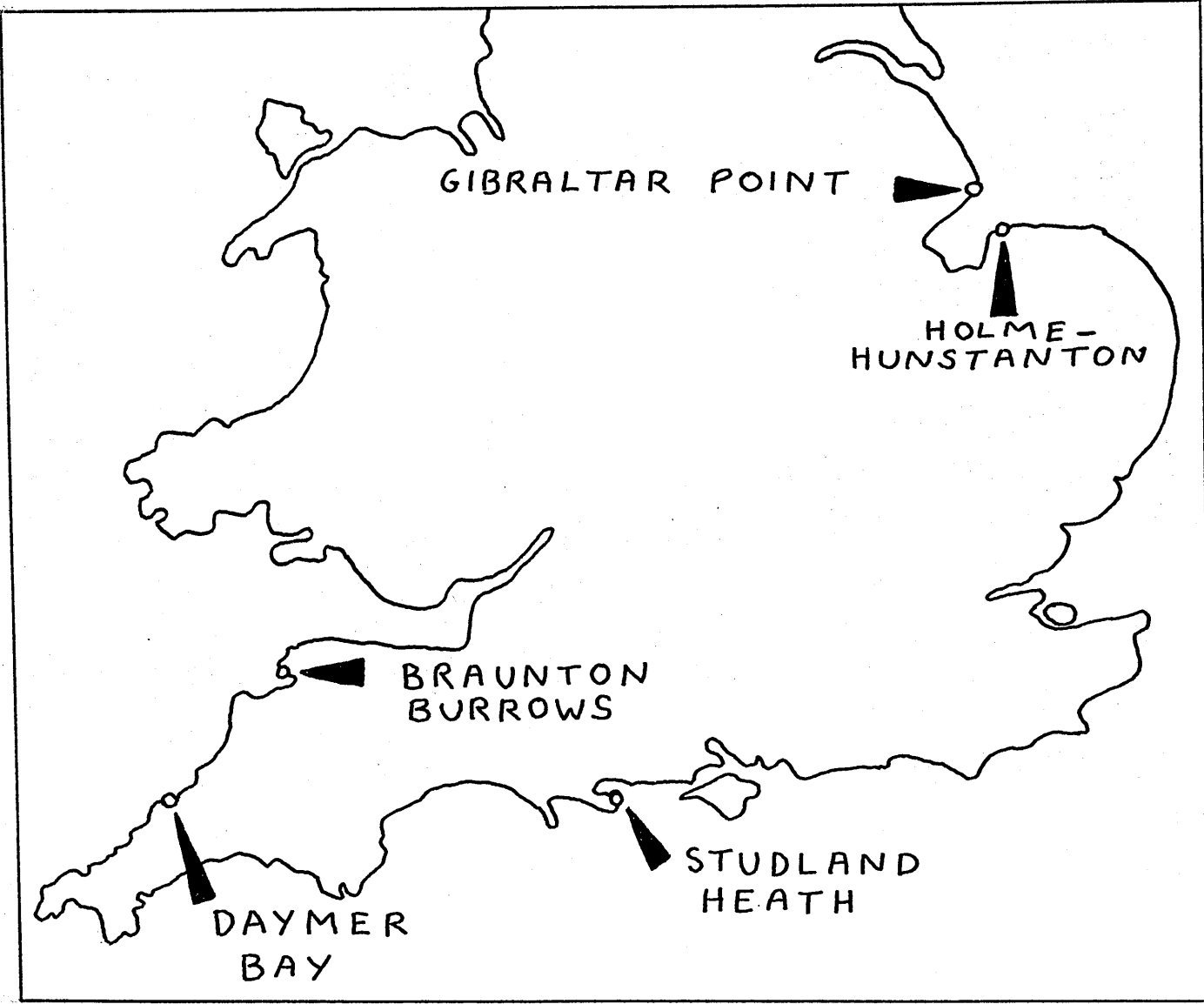
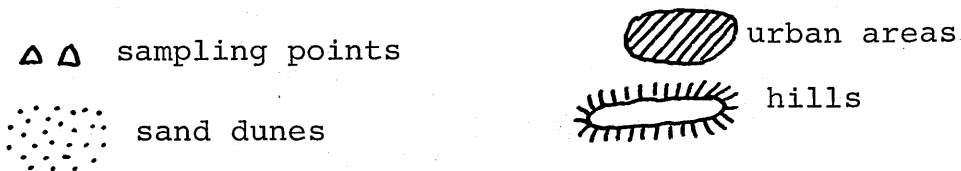
1 g dry soil contains  $\frac{14}{10^3} \times \frac{KS}{W_d}$   $\mu\text{g}$  nitrogen as nitrate

or

$$[\text{N}] \text{ as } \text{NO}_3^- = \frac{14}{10^3} \times \frac{KS}{W_d}$$

Figure 2.2    Position of four subsidiary locations for study of sand and dune soils

Key for large scale maps 1:50,000



## 2.4 Subsidiary Study Areas - Experimental Details

Although the dunes of the Hunstanton-Holme area formed the principal site for the long-term study a number of other subsidiary locations were also examined using the same sampling methods and the same analytical techniques. The objectives were:

- (i) To compare the levels of dune-soil nitrogen at Hunstanton-Holme with similar types of dune localities.
- (ii) To compare the dune systems supporting *Hippophaë* scrub with those lacking *Hippophaë*.
- (iii) To discover if litter breakdown on dunes contributes much nitrogen to the soil.

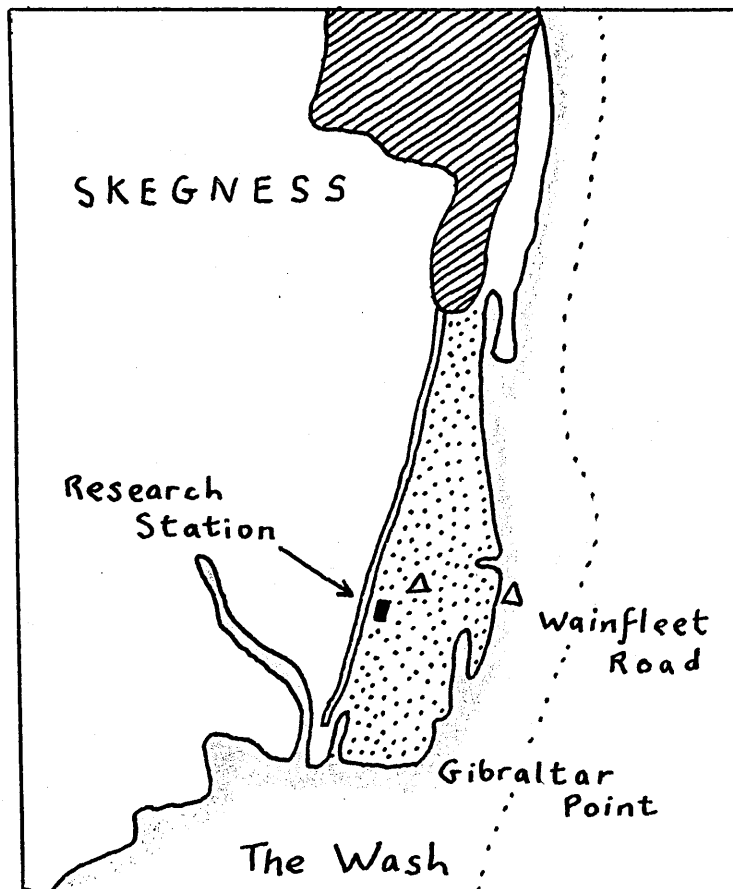
Four sites were chosen, two on the west coast, one on the south coast, and one on the east coast. This made it possible to contrast "carbonate dunes" of the east, and south-east coasts, with the "acid dunes" of the west coasts. This particular distinction was demonstrated by Salisbury's studies on the dunes of Blakeney, Norfolk, and Southport, Lancashire. (SALISBURY, 1922, SALISBURY 1925).

### 2.4.1 East Coast Gibraltar Point, Lincolnshire

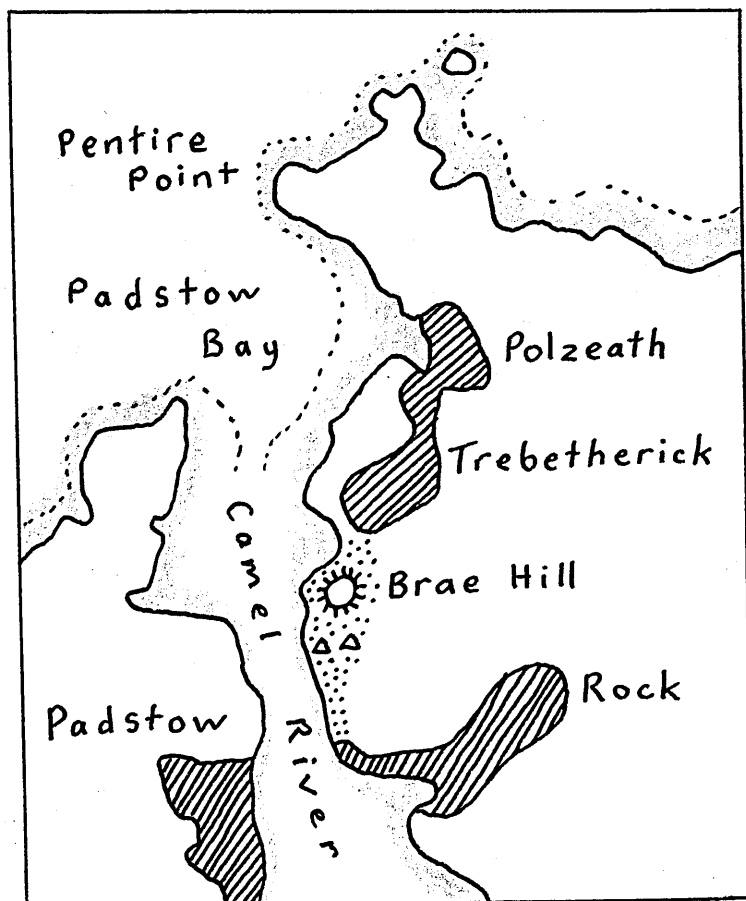
(O.S. Grid Reference TF 565594)

Gibraltar Point is a headland which marks the western edge of The Wash. It lies directly opposite the Hunstanton-Holme area in a north-westerly direction and the distance is about twenty kilometres across the Wash, so that in clear weather the Point is visible on the horizon from the dunes

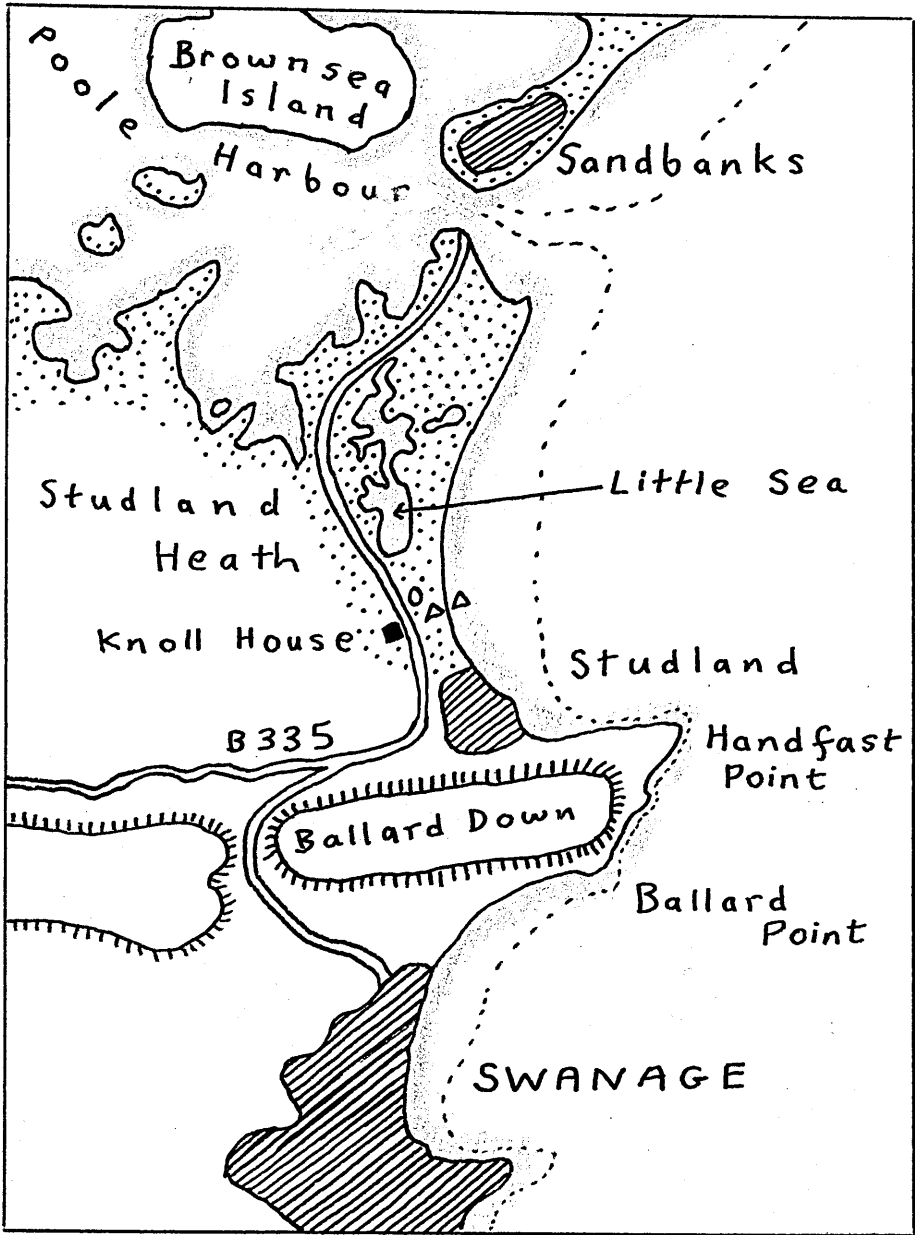
Map 2.5 Gibraltar Point, Lincolnshire  
O.S. Grid Ref. TF 565594



Map 2.6 Daymer Bay, Cornwall  
O.S. Grid Ref. SW 927768

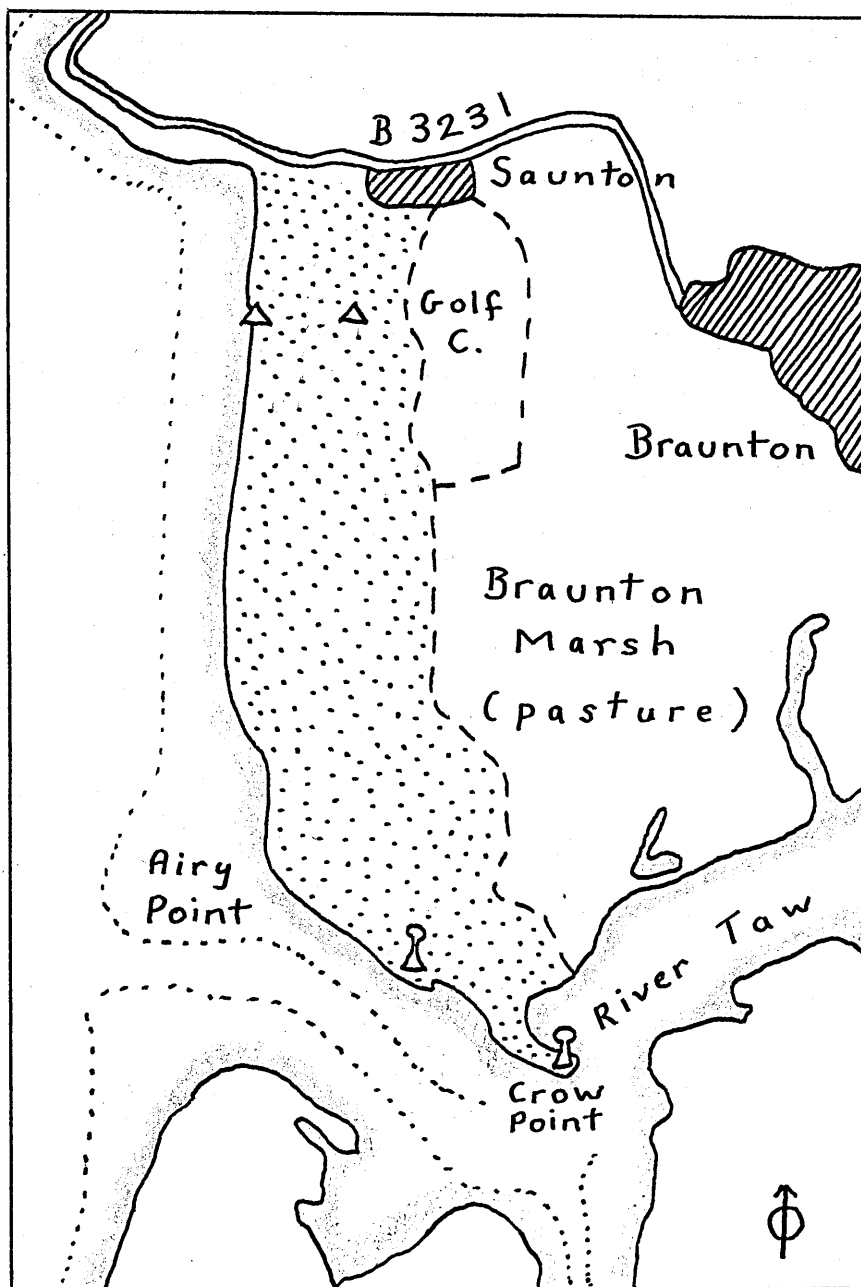


Map 2.7 Studland Heath, Dorset  
O.S. Grid Ref. SZ 034 836





Map 2.8 Braunton Burrows, Devon  
O.S. Grid Ref. SS 450 350



of Holme. Geologically the area is much like that of the Norfolk coast. However while Gibraltar Point lies on lower greensand and clays, Holme is directly on the main chalk. The Point itself is composed of shingle and beach sand of recent origin. There is the typical pattern of dune ridges running parallel to the coast, and there is a wide gently shelving beach. At the springs the tide goes out, in some places for a distance of eighteen kilometres.

Much of the dune system is heavily overgrown with sea-buckthorn which forms an impenetrable scrub up to 5 metres high in places. At present the area is a Nature Reserve owned and managed by the Lincolnshire Naturalists' Trust.

Fifteen samples were collected at this location; five replicates from the beach above the high tide mark, five from under buckthorn scrub of medium age (4-6 yrs) and five from beneath older scrub (10-14 yrs). The samples were collected in July 1981, and analysed within two days of the field visit.

#### 2.4.2 South Coast Studland Heath, Dorset

(O.S. Grid Reference SZ 034 836)

Studland Heath is a low peninsula bounded on the south by the chalk ridge of the Isle of Purbeck, on the east by Studland Bay, and on the north by Poole Harbour. Geologically the area is composed of Eocene clays and gravels abutting the main chalk of the Purbeck ridge. The whole peninsula is of very recent origin, having evolved during the last four centuries, as indicated on historical maps (DIVER 1933, DIVER & GOOD 1934, DIVER 1936).

A series of dune ridges run approximately north-south parallel to the beaches of Studland Bay enclosing several fresh water lagoons to the north of the Peninsula. These are typically "acid dunes" in that they support a flora which is lime-hating and which prefers neutral or acid soil conditions, notably heather, ling and bracken. Gorse is also common, but sea-buckthorn is absent from the area.

Twenty samples were collected from the southern end of the Nature Reserve about four hundred metres from the car park near Knoll House. Five replicates were taken from the beach just above the high water mark, five were taken from young marram dunes, and five from the heather scrub. The samples were collected in April 1981.

#### 2.4.3 West Coast (i) Daymer Bay, Cornwall

(O.S. Grid Reference SW 927 768)

Daymer Bay is a small inlet on the eastern shore of the Camel Estuary which runs north-south at this point. It is about one and a half kilometres north-east of the town of Padstow. The area geologically is Devonian sandstone, and Daymer Bay lies on the Upper Old Red Sandstone. Dunes are common on both shores of the estuary but none are very extensive. At Daymer Bay there are two series of dunes separated by the bluff of Brae Hill. The samples were collected to the south of this hill in a variety of sites. Twenty soil samples were collected; five replicates from the sea beach above the high tide mark, five from young marram dunes, five from a dune slack and five from a hawthorn scrub which had established itself on the dunes.

This was a particularly interesting location because the dune slack contained at least two legumes, *Lotus corniculatus* (Bird's Foot Trefoil) and *Anthyllis vulneraria* (Kidney Vetch) which might be nitrogen-fixers and capable of enhancing soil nitrogen levels. The hawthorn scrub contained a rich flora and a significant litter, and again here was a useful opportunity to measure the nitrogen levels below a scrub lacking sea-buckthorn. There was no buckthorn present in the entire area. A fuller list of the plant species observed is given under the experimental results.

West Coast      (ii)    Braunton Burrows, Devon  
                         (O.S. Grid Reference SS, 450 350)

Braunton Burrows is a very extensive area of dunes lying north of the mouth of the Taw-Torridge Estuary. It is about four kilometres west of the village of Braunton, and about ten kilometres west of Barnstaple. The Burrows are about  $1\frac{1}{2}$  by 5 km, a series of parallel dune ridges running approximately north-south facing Barnstaple Bay.

Geologically the dunes lie over the Culm measures, and in the north they adjoin the cliffs of Old Red Sandstone at Saunton Down.

Twelve years ago there were extensive stands of sea-buckthorn on the Burrows, principally in the north, around the vicinity of the car park below the cliffs. A policy of eradication had been carried out by the staff of the Reserve assisted by volunteer corps. Consequently in 1981 the sea-buckthorn had almost disappeared.

After a careful search of the dune hollows in the north a few plants of sea buckthorn were discovered, mostly young plants less than half a metre in height, but propagating beneath the sands. One larger plant over a metre high was also found. Eleven soil samples were taken, five replicates on the sea beach above high tide, five from beneath the young buckthorn and one from beneath the large medium-age plant.

The samples were collected in April 1981.

Chapter Three: Sources of error -  
a summary of the problem encountered and  
the precautions taken.

A series of checks and controls were used in this study. They are summarised in the table below. In the context of this study a check is a simple test to give a yes or no answer; a control is either a series of replicates to indicate the extent of variation, or a graded series of tests to find a graded response.

Experimental component	Check (CH) or control (CO)
1. Buckthorn scrub sites	
1.1 problem of defining the age of sea-buckthorn bushes	CO measurement of stem diameter on two occasions and measurement of bush height
1.2 comparison of three different age-groups	CO series of ten replicates of each age group
2. Effects of litter on soil nitrogen levels	
2.1 possible contribution of leaf litter	CO five controls and five experimental plots for each age group
2.2 effectiveness of polythene sheet barrier	CO carbonate content of control and experimental sites CO phosphate content of control and experimental sites CO five beach samples
3. Collection of soil samples at depth - variation of nitrate with depth	CO graded series of soil samples at different depths - measured for nitrate
4. Drying samples of soil - assessment of dry weight of wet-extracted sample	CO six replicates to indicate the percentage water content variation

Experimental component	Check (CH) or control (CO)
5. Extraction of soluble materials from soil - effectiveness of the water-extraction method	CO comparison of aqueous and chloride extraction procedures using 15 replicates
6. Analysis for nitrate ion	
6.1 inaccuracy of comparator	CH use of standard solutions
6.2 problem of haze	CH improved filtration
6.3 charring on evaporation	CH investigation of spectrum and control of methods
6.4 stability of NPDSA	CH examination of solutions at long time intervals
6.5 calibration of spectrophotometer by standards	CO preparation of standard nitrate solutions
6.6 choice of wavelength	CH examination of NPDSA spectrum
6.7 choice of suitable reference solution	CH examination of suitable solutions
6.8 drift on spectrophotometer	CH use of reference solution in test cuvette
6.9 possible contamination of reagents	CH use of blank solutions
6.10 interference by chloride in test solutions	CO measurement of chloride present in solution
7. Analysis for ammonium ion	
7.1 problems with Nessler's reaction	CO standard ammonium solutions used with comparator CH quite unsatisfactory in spectrophotometer
7.2 use of indophenol reaction	CH very satisfactory in spectrophotometer
7.3 impurity of reference solutions and blanks	CH ammonia tested for and found - invalidates the assay



Experimental component	Check (CH) or control (CO)
8. Analysis for phosphate ion	
8.1 modified Denigès reaction	CO standard solutions assayed
8.2 contrast between February and October	difficulty unresolved

### 3.1 Sea-Buckthorn Scrub Analysis

There were two problems initially in the selection of buckthorn scrub sites, one was to obtain a representative sample, and the other was to establish the age of the individuals chosen. As the scrub was spread over a distance of about a kilometre in a band about 30 m wide a place was chosen in the centre of the range to be the maximum distance from the two points of public access, at Holme beach, and Old Hunstanton.

A method of selection by quartering and random number tabulation was considered, and rejected. Given the impenetrable nature of mature buckthorn scrub, the practical consideration of easy accessibility for soil sampling was the paramount one. Consequently scrub sites were chosen on the periphery, clear of other bushes. Three age-groups were selected, on the basis of their general size, as indicated in the accompanying illustrations. To limit the collection and analysis of soil samples to manageable proportions, ten replicates in each age group were used. The distribution of these replicates is shown in the map. (Map 2.4)



Young Hippophaë plants  
on the dune top



Medium age Hippophaë plants among marram grass  
on the landward side of the dune





Old-age Hippophaë plants. The ranging pole is marked off in half-metres

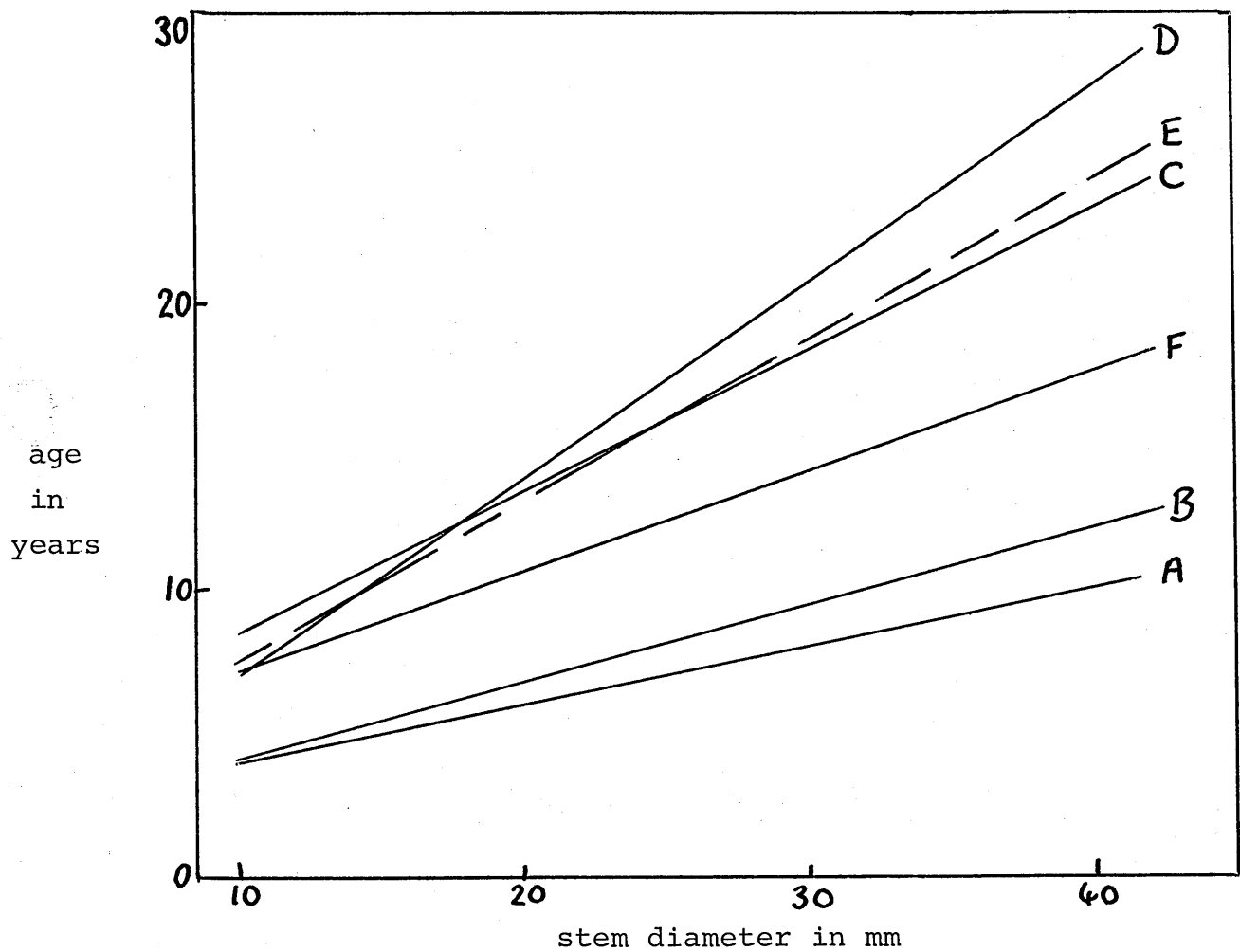


General view of the sea-buckthorn colony looking towards the beach huts at Holme Beach car park

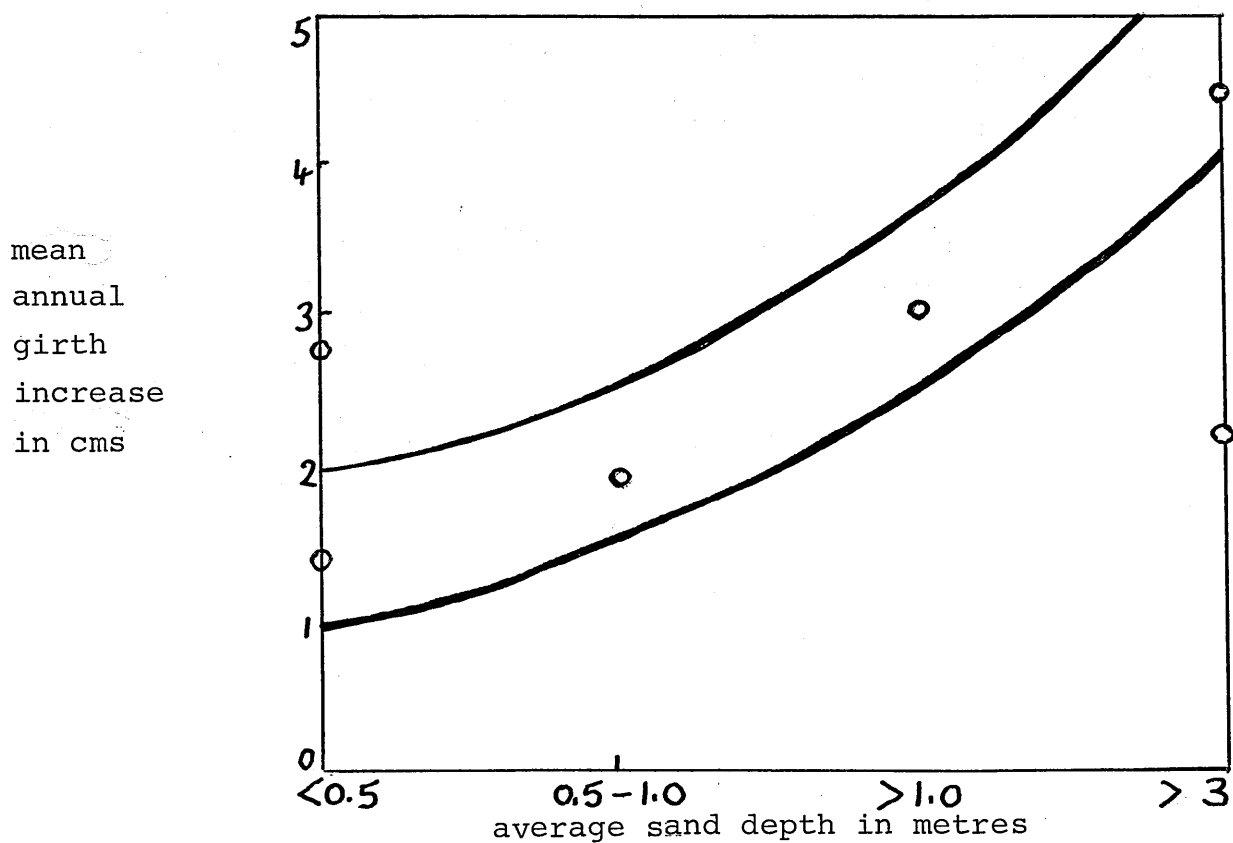
It was necessary to mark the bushes with anodised aluminium labels, low down for ease of later identification. Those on the bare sand ridge nearest the sea were continually covered by drifting sand, and the labels were buried, and proved difficult to find even with the help of the 1:50 scale map. An additional marker (a section of coarse nylon fishing net) was used, as it was tough and readily visible, but not so unusual as to excite the interest of the general public. One anodised label disappeared, so that site B could not be identified from September onwards.

It was known that nodulation in sea buckthorn increased with age to a maximum at about 10-12 years (STEWART & PEARSON 1967) and levels of inorganic nitrogen also followed this pattern. Consequently buckthorn bushes were chosen in three groups - young (2-3 years), medium (4-5 years) and old (11-14 years) to see if this trend was identifiable in the seasonal measurement of nitrogen levels.

In the pilot study an attempt to date buckthorn scrub was made by cutting stem sections and counting the annual rings in a phloroglucinol-stained thin section. However the centre of the stem is somewhat compacted and the identification of early rings is very difficult. Neither was this practical in the main study because it would have involved damage to the oldest mature bushes. An alternative approach was to take measurements of stem diameter or total height and to use these to calculate approximate age using the regression lines or equations from a study of buckthorn scrub on Spurn Peninsula (LEDWOOD and SHIMWELL 1971). However the data indicated a considerable degree of



Graph 3.2      Variation of girth increase with depth of sand (data from Ledwood and Shimwell 1971).



variation (Table 3.1) in the stem diameters of plants of the same age. For example, at one site 10 year-old bushes had stem diameters ranging from 25 to 40 mm. A further complication was provided by the fact that the regression lines varied from one population to another, even within a comparatively small area like the Spurn Peninsula (Graph 3.1). A plant with a 30 mm stem diameter might be anywhere between 8 and 21 years old depending upon the site.

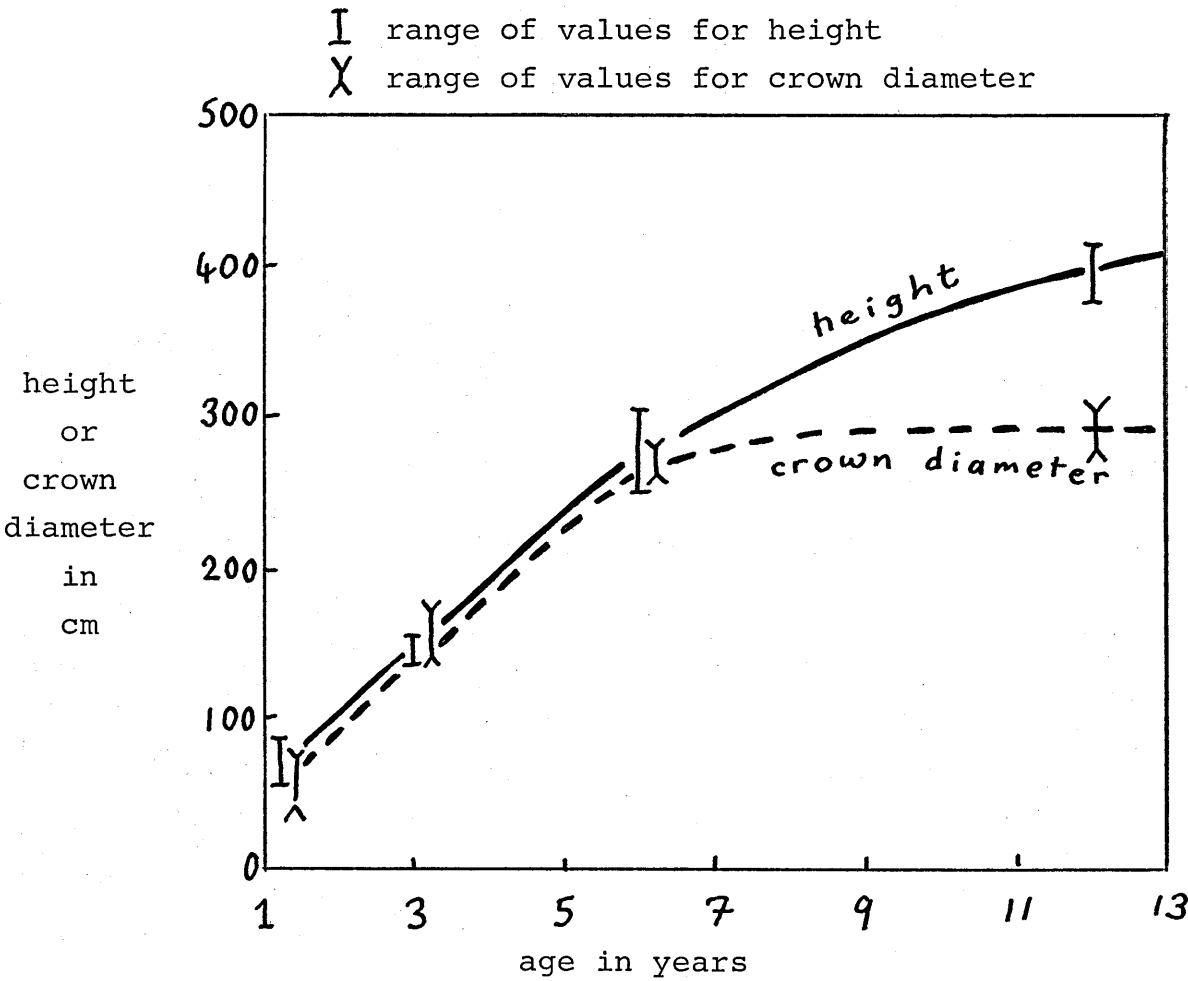
Ledwood and Shimwell tried to relate the mean annual increase in girth to the depth of sand at the different sites. Although there seems to be a positive relationship so that the deeper the sand the greater the increase in stem diameter the variation is still considerable (Graph 3.2).

A study of buckthorn on sands of the Don River in the Soviet Union (VASCHENKO 1973) made use of a wider range of parameters in association with age. Height of bush, diameter of crown, circumference of stem, and annual shoot increment were measured, but in each case a range of values is quoted (Table 3.2). No statistical data or regression analysis is indicated, but it seems clear that none of the parameters measured give anything more than an approximate idea of the chronological age. (Graphs 3.3 and 3.4).

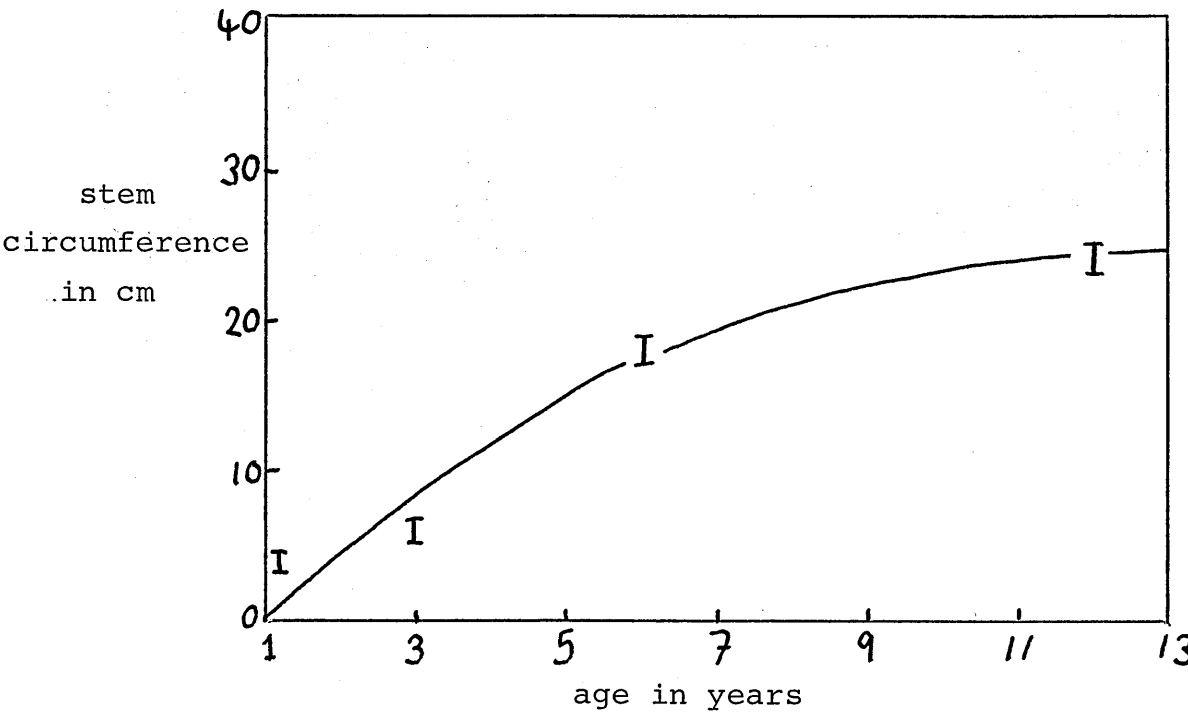
Some of Vaschenko's data on shoot growth show an irregular pattern of increase from year to year, as if climatic variation might be involved. The general trend observed was a decline in shoot growth with increasing age of bush. (Graphs 3.5 and 3.6).

Taking into account all of this variation it seemed to be advisable to do no more than nominate an approximate range

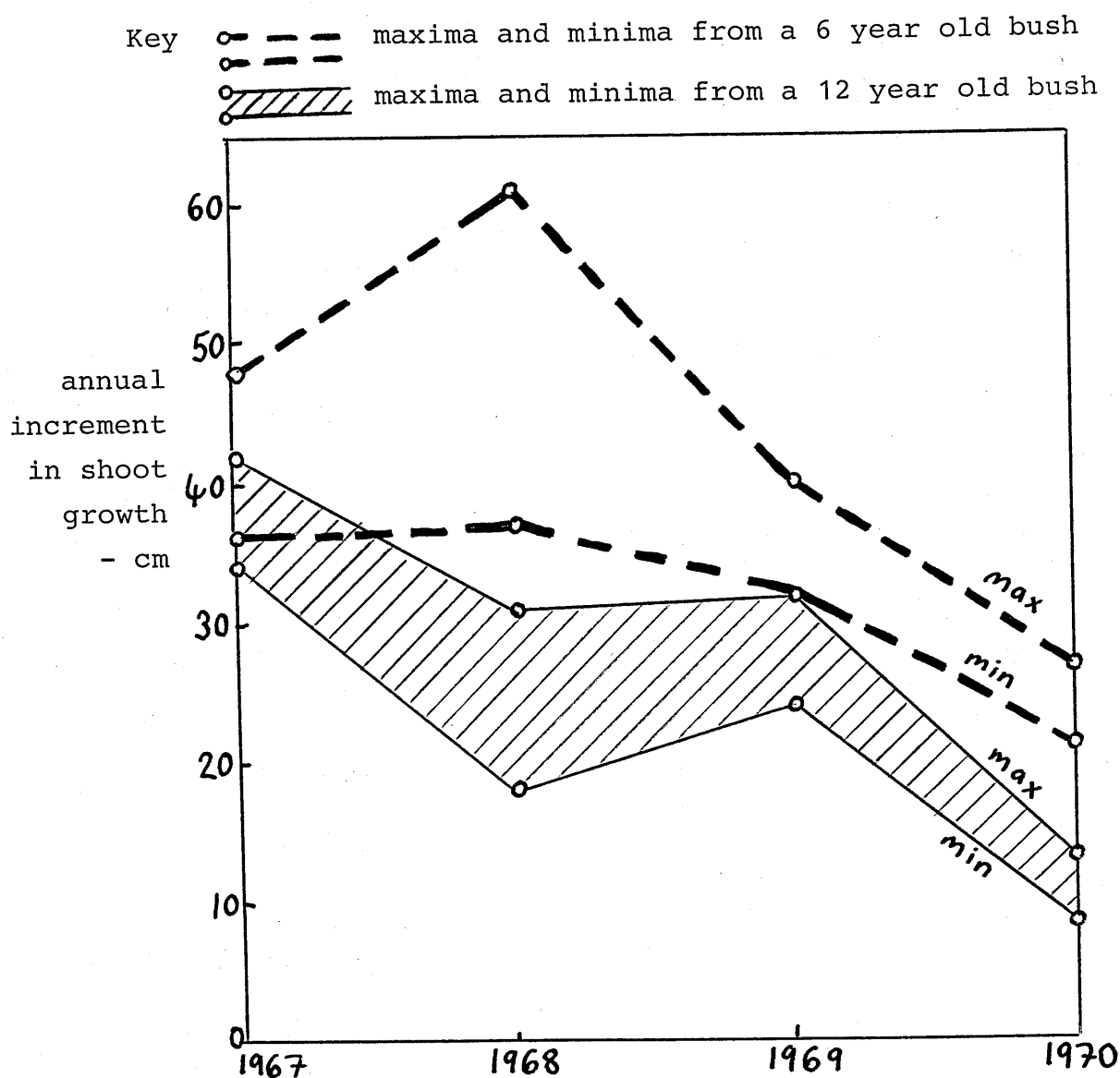
Graph 3.3    Growth of Hippophaë with age (data from Vaschenko 1973).



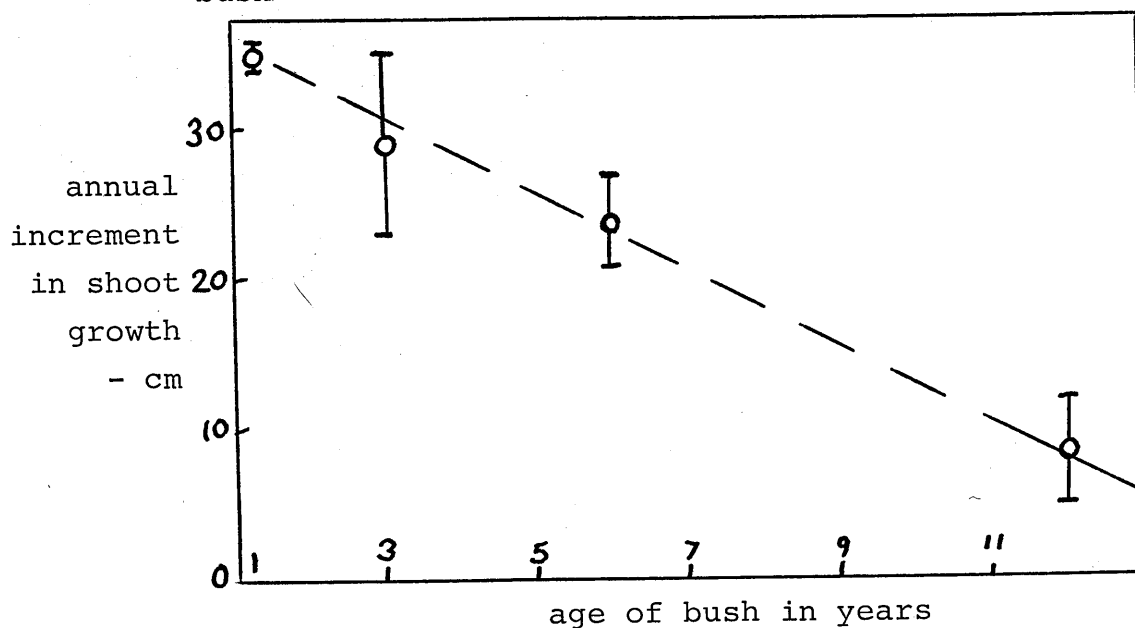
Graph 3.4    Increase in stem-circumference with age in Hippophaë (data from Vaschenko 1973)



Graph 3.5 Shoot-growth variation during a three year period

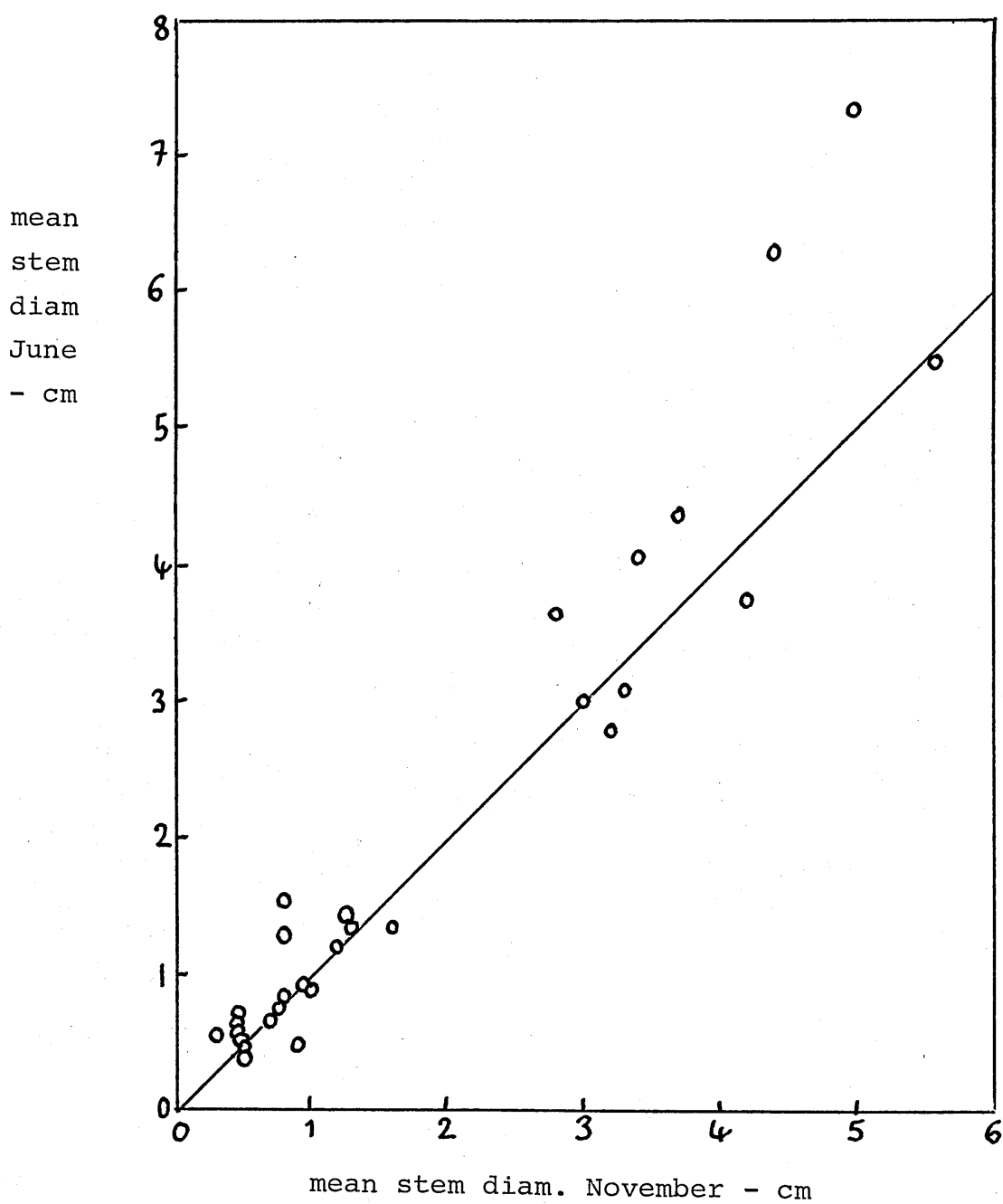


Graph 3.6 Shoot-growth variation with the age of Hippophae bush





Graph 3.7 Assessment of accuracy in measurement - comparison of stem diameter data on two different occasions



of ages for the three groups of buckthorn, young, medium and old which were investigated at the Hunstanton-Holme location.

The data on stem diameters of the buckthorn scrub are given in Table 3.3, along with the heights. The irregularity of the stems creates problems in finding an accurate mean diameter. The agreement between the measurements made in June and in November are not as good as might have been expected; a further reason for avoiding an attempt to estimate ages accurately on the basis of such data. (Graph 3.7).

Table 3.1 Age and stem-diameter of sea-buckthorn  
(data from Ledwood and Shimwell 1971)

Table of regression lines for six sites

SITES SD	A Point dunes	B Kilnsea warren	C Chalk Bank E	D Chalk Bank W	E Chalk Bank Pr.	F Greedy Gut shin.
10	3.9	4.0	8.3	7.0	7.4	7.2
30	-	-	-	-	18.5	14.0
40	10.0	12.0	23.2	27.5	-	-

SD = stem diameter in mm.

Figures in the cells are corresponding ages in years.

Table 3.2 Growth rates in samples of sea-buckthorn  
(data from Vaschenko 1973)

Age years	Height cm	Crown Diameter cm	Stem Circum- ference cm	Annual increase in growth of shoots in cm			
				1970	1969	1968	1967
1	55-85	42-73	3.5-4.5	34-36	-	-	-
3	135-153	144-176	5.1-6.8	23-35	43-73	42-67	-
6	250-305	265-277	17-19	21-27	32-40	37-61	36-48
12	372-412	278-301	24-26	5-12	20-26	14-25	33-36
12*	501	301-312	33	7-13	24-32	18-31	34-42

\* relatively large tree

NB stem circumference was measured at a height of 30 cm  
from ground level.

Table 3.3 Stem diameter and height of sea-buckthorn bushes  
Hunstanton

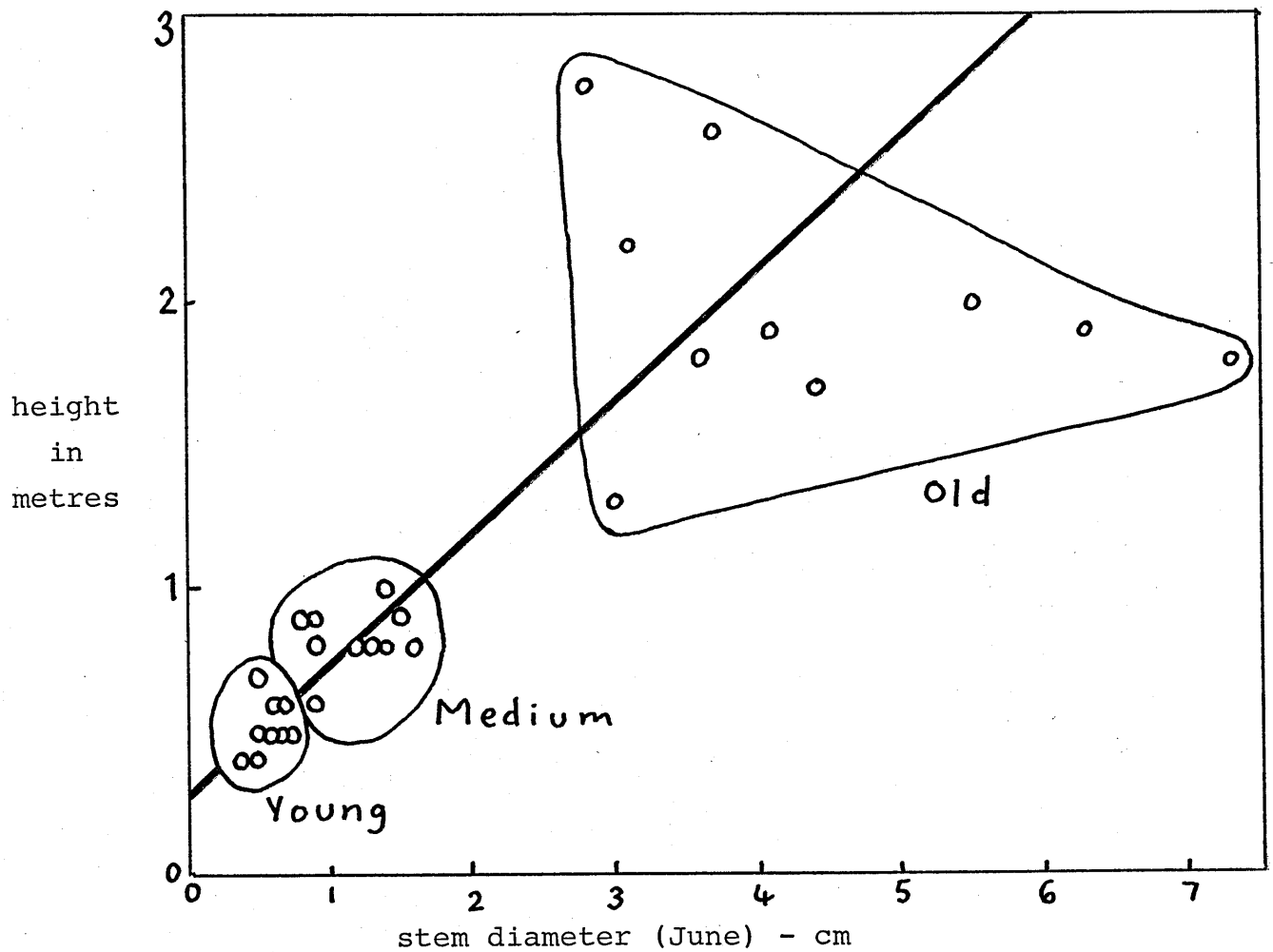
		Control Sites			Experimental Sites		
		SD-J	SD-N	H-O	SD-	SD-N	H-O
Young		0.5	0.5	0.7	0.5	0.5	0.4
		0.6	0.3	0.5	0.5	-	-
		0.7	0.4	0.6	0.5	0.9	0.5
		0.6	0.5	0.6	0.7	0.7	0.5
		0.6	0.5	0.5	0.4	0.5	0.4
Medium		1.2	1.2	0.8	1.4	1.3	0.8
		1.5	1.3	0.9	0.9	1.0	0.8
		0.8	0.8	0.9	1.3	0.8	0.8
		1.4	1.6	1.0	1.6	0.8	0.8
		0.9	0.9	0.9	0.9	0.8	0.6
Old		4.4	3.7	1.7	2.8	3.2	2.8
		6.3	4.4	1.9	3.7	4.2	2.6
		7.3	5.0	1.8	3.1	3.3	2.2
		3.0	3.0	1.3	4.1	3.4	1.9
		5.5	5.6	2.0	3.6	2.8	1.8

SD-J= stem diameter in cm      June


SD-N = stem diameter in cm - November

H-O = height in meters -      October

Graph 3.8 Correlation between height and stem-diameter in Hippophaë bushes. (data from Hunstanton-Holme)



Graph 3.9 Comparison between Hippophaë in UK and in USSR for height/stem diameter correlation.

Key  Vaschenko data

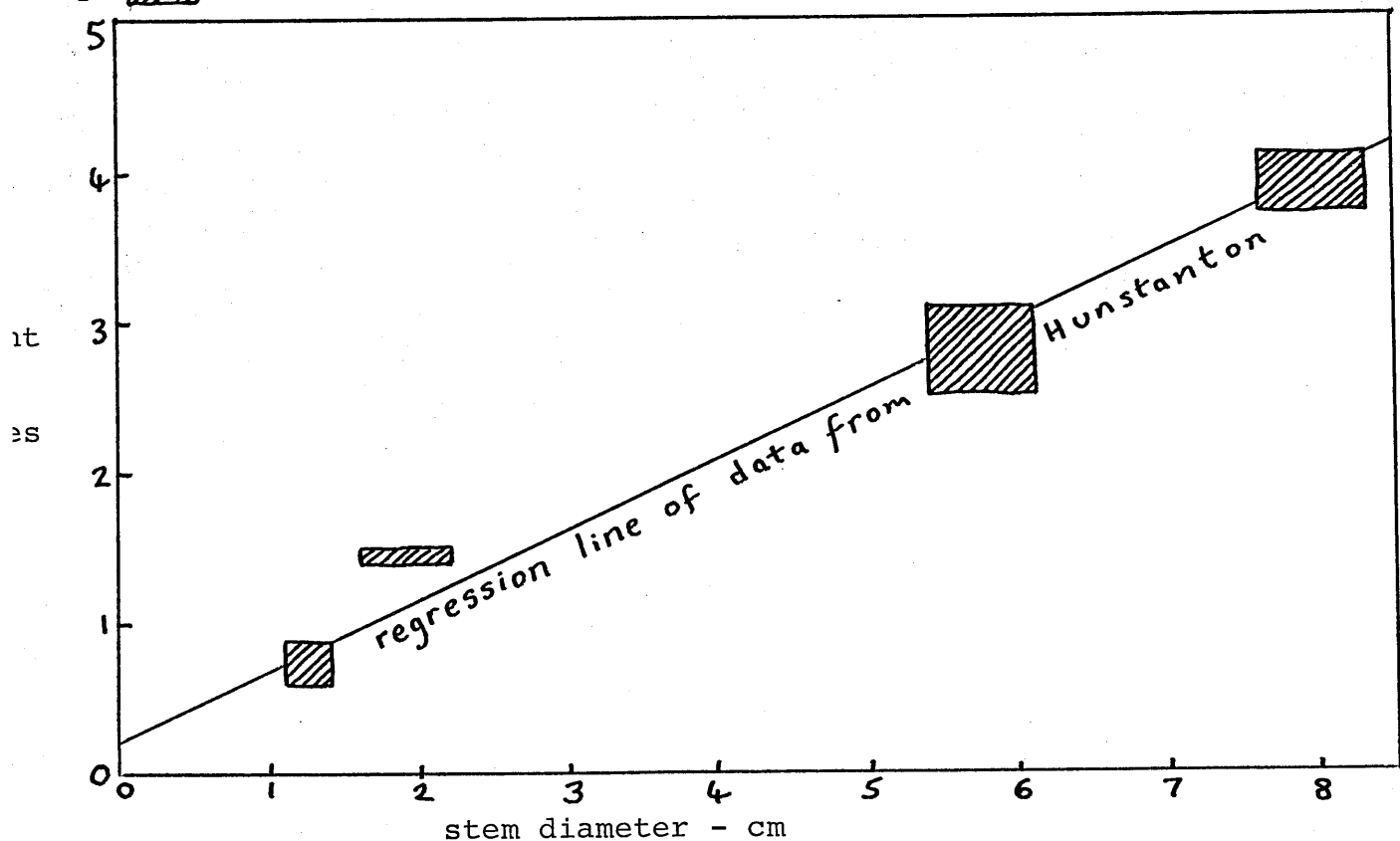


Table 3.4 Correlation between height and stem diameter in sea buckthorn (data derived from Vaschenko 1973)

stem diam $\frac{C}{\pi}$ cm	1.1-1.4	1.6-2.2	5.4-6.1	7.6-8.3	10.5
height metres	0.6-0.9	1.4-1.5	2.5-3.1	3.7-4.1	5.0

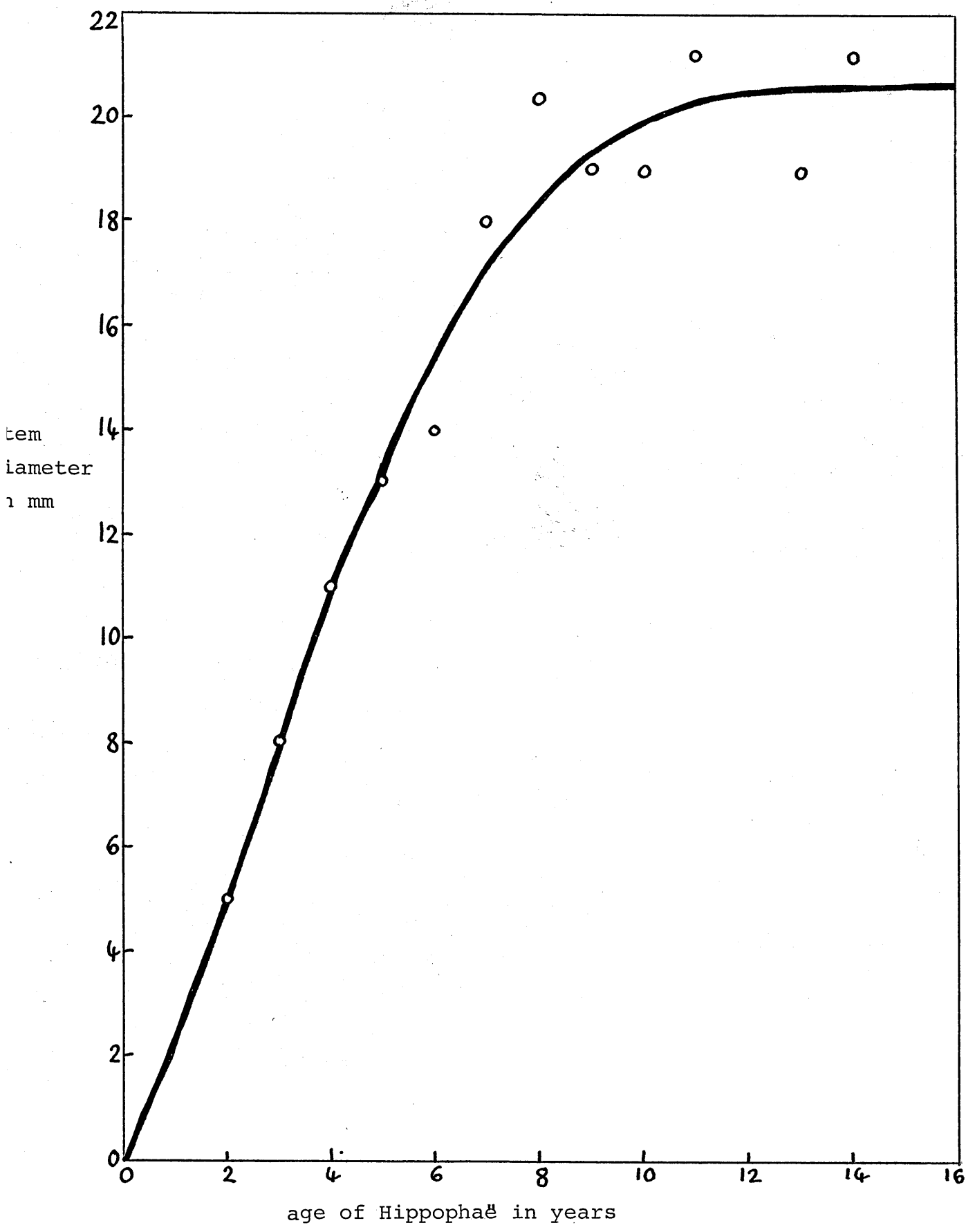
Hunstanton regression line	st. diam cm	0	6.0
	height m	.25	3.0

An examination of the relation between height and stem diameter shows a limited degree of correlation. The variation is greatest in the older bushes (Graph 3.8). A re-examination of the Vaschenko data was made, converting the stem circumference values to stem diameter values assuming that the relationship  $\text{diameter} = \frac{\text{circumference}}{\pi}$  is approximately true.

These data are given in Table 3.4. The regression line from the Hunstanton data fits the Vaschenko data very well, (Graph 3.9) indicating the close similarity between the form of buckthorn in maritime habitats in the U.K., and continental habitats in the U.S.S.R.

A further complication emerged from a study of the sea-buckthorn on the Dutch Frisian islands, (DE VRIES 1947). As the amount of humus in the soils increased, and the pH grew more acid the levels of lime began to decrease and this correlated with a decline in rate of increase of the stem diameter. The relationship between age and stem diameter was shown to be a curvilinear one (Graph 3.10). The leveling-out begins to occur at about 7 years and according to

Graph 3.10 Correlation between stem-diameter and age in Hippophaë (data from De Vries 1947)



de Vries is due to the symbionts of the root nodules which prefer alkaline conditions. It is interesting to note that Vaschenko's data (Graphs 3.3 & 3.4) also show a levelling out at seven years, with respect to crown diameter and also stem diameter. Though no information on pH changes is available there is an interesting correlation here with the data of de Vries.

The curve in Graph 3.10 is based on a series of modes of normally distributed frequencies of stem diameters for each year group. As with Ledwood and Shimwell's data there is considerable variation such that a 10 mm stem would indicate an age between 3 and 6 years.

The problem with much of this type of data is that although it reveals trends which are true on a statistical basis it does not enable an accurate statement to be made about one individual instance. Consequently sample sites were described as "young", "medium" and "old", and an approximate age was given for each based upon Ledwood and Shimwell's data. The mean stem diameter for each of the six groups was calculated and using regression lines derived from Ledwood and Shimwell's figures the appropriate age could be read off. As mentioned earlier these authors had obtained data which yielded six regression lines, so the one chosen was from a habitat which corresponded closely with that at Hunstanton-Holme, A on Graph 3.1.

The age data are given in Table 3.5.

Table 3.5 Derivation of mean ages of sea-buckthorn from stem diameter measurements

	Mean Stem Diameter mm		Approximate Age - Years	
Age Group	Control	Exp	Control	Exp
Young	5.2	5.8	2½	3
Medium	11.6	10.8	4	4½
Old	48.2	34.2	9	11



### 3.2 Effects of litter on soil nitrogen levels

Any attempt to demonstrate the importance of nitrogen-fixing colonizers like sea-buckthorn must distinguish between the contribution made by the break-up of ageing buckthorn root-nodules, and the contribution from the degradation of normal litter on the soil surface.

The use of control and experimental sites (five replicates of each for the three age-groups of sea-buckthorn scrub) attempts to separate the two contributions to soil nitrogen. The control sites were left in their normal state, while the experimental sites were prepared with a one metre square polythene sheet 10 cm below the soil surface.

If litter decomposition makes a measurable contribution to dune soil nitrogen in the latter stages of the experiment there should be a distinct difference between control and experimental sites in terms of nitrogen levels. Failure to demonstrate a significant difference would indicate that the effect of litter breakdown was marginal, in this particular habitat.

Similarly any evidence of very low nitrogen levels in the dune soil beneath non-nitrogen-fixing scrub would tend to confirm the validity of the absence of difference between control and experimental sites. It would provide further proof of the hypothesis that dune vegetation litter makes only a minimal contribution to nitrogen levels.

These two ideas are examined in detail within Chapter 4 (Analysis of Results). One further series of control experiments were required to assess the effectiveness of the

polythene sheet as a barrier to downward percolation in the experimental sites. Evidence of differences between control and experimental sites, in factors other than nitrogen, would help to confirm that the sheets presented a sufficiently impervious barrier.

Table 3.6 shows the level of carbonate in dune soil as a percentage of the dry weight. These soil samples were taken in October, towards the end of the experiment, when the likelihood of distinct differences would be greater.

Carbonate content was estimated by treating weighed, dried soil samples (about 100-150 g of each) with 1 M hydrochloric acid for 12 hours. The samples were washed with de-ionized water for 24 hours, and dried at 80 °C for 24 hours, before weighing once more. A visual inspection of the samples indicated a high level carbonate as quantities of mollusc shell fragments were present.

The levels of carbonate varied between 1.4% and 6.3%. Although the results were reasonably consistent, there was some degree of variation about the mean value. Table 3.7 gives some idea of this variation in terms of standard deviation, and standard error. Graph 3.11 indicates the general trends shown by the mean values of carbonate, and the standard error gives some idea of the reliability of the differences.

Graph 3.11 indicates that the level of carbonate declines with the increasing age of the buckthorn scrub. The accepted mechanism is thought to be determined by the increase in the organic content of the soil litter with the age of the

dune. Decomposition of organic matter in litter produces various organic acids which reduce soil pH, and dissolve carbonates. It was very obvious that the organic content of the soil samples increased from young through medium to old sea buckthorn scrub. No quantitative data are available however, mainly because this was only a subsidiary experiment and there were considerable problems in separating the dark organic fraction of the soil samples.

The detailed study of the soils of nearby Blakeney Point (SALISBURY 1922) amply demonstrated the relationship between the age of the dune, the organic content of the soil, the pH and the carbonate content. The examination for carbonate in this study is used solely as a discriminator between control and experimental sites.

In this particular context Graph 3.11 indicates very little difference between experimental and control sites given the

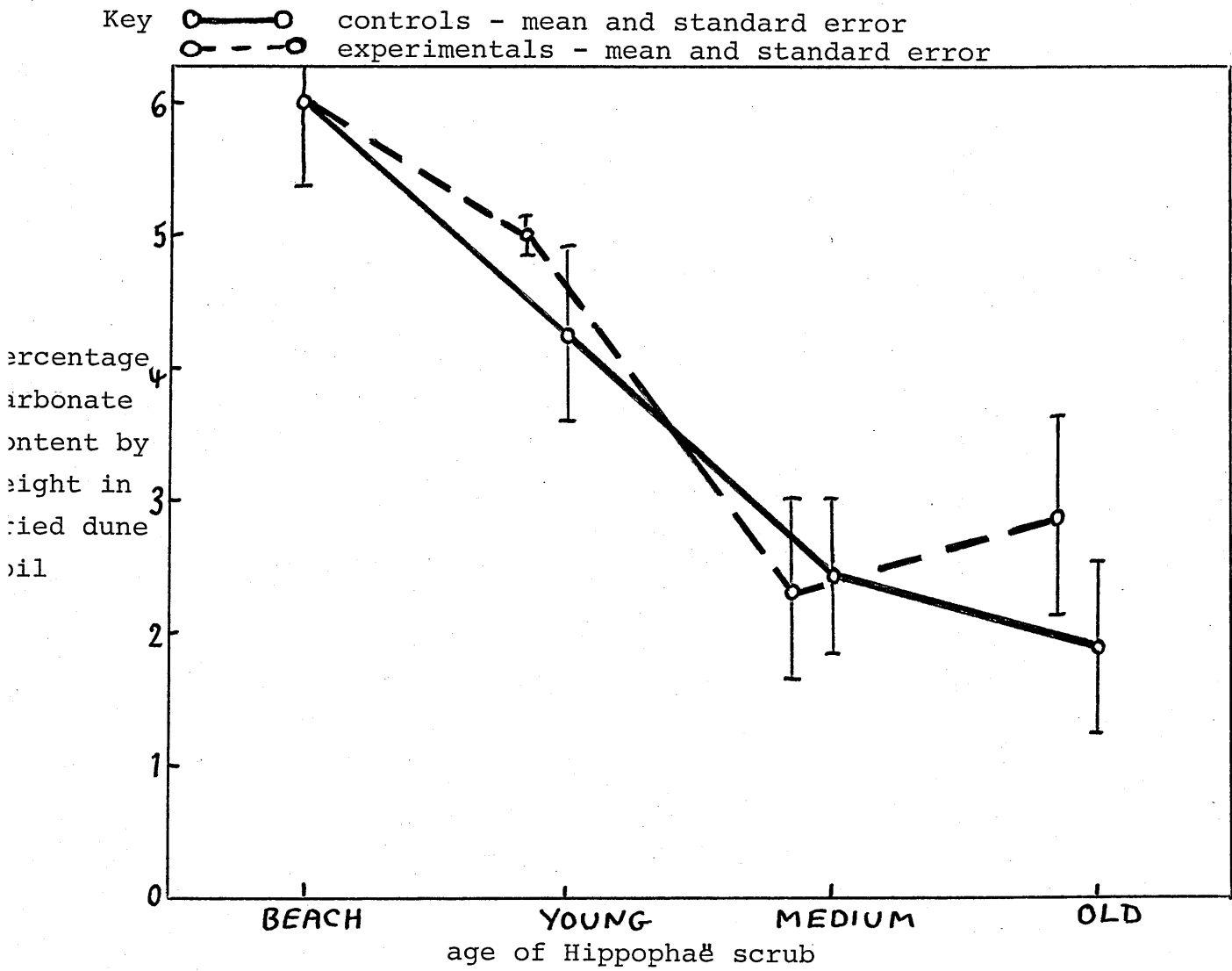
Table 3.6 Percentage weight of carbonate in dune soil -  
(raw data - October 1981 Hunstanton-Holme)

Sites	Replicates				
Beach	6.3	6.4	6.2	4.9	6.0
YC	3.9	3.8	4.4	5.1	4.1
YE	5.1	-	5.1	4.8	5.0
MC	2.3	2.2	2.5	3.1	2.1
ME	2.6	2.8	1.4	2.4	2.4
OC	1.7	2.4	2.3	1.4	1.8
OE	2.2	3.2	3.5	2.3	2.3

Key : see Table 3.7

extent of the standard error of the means. It does not appear that the use of a barrier at the experimental sites was producing any change in the level of carbonates, at that stage in the experiment. Whether this would create a bigger

Graph 3.11 Carbonate content of dune soils - data from Hunstanton



Graph 3.12 Carbonate content of dune soils - data from subsidiary locations

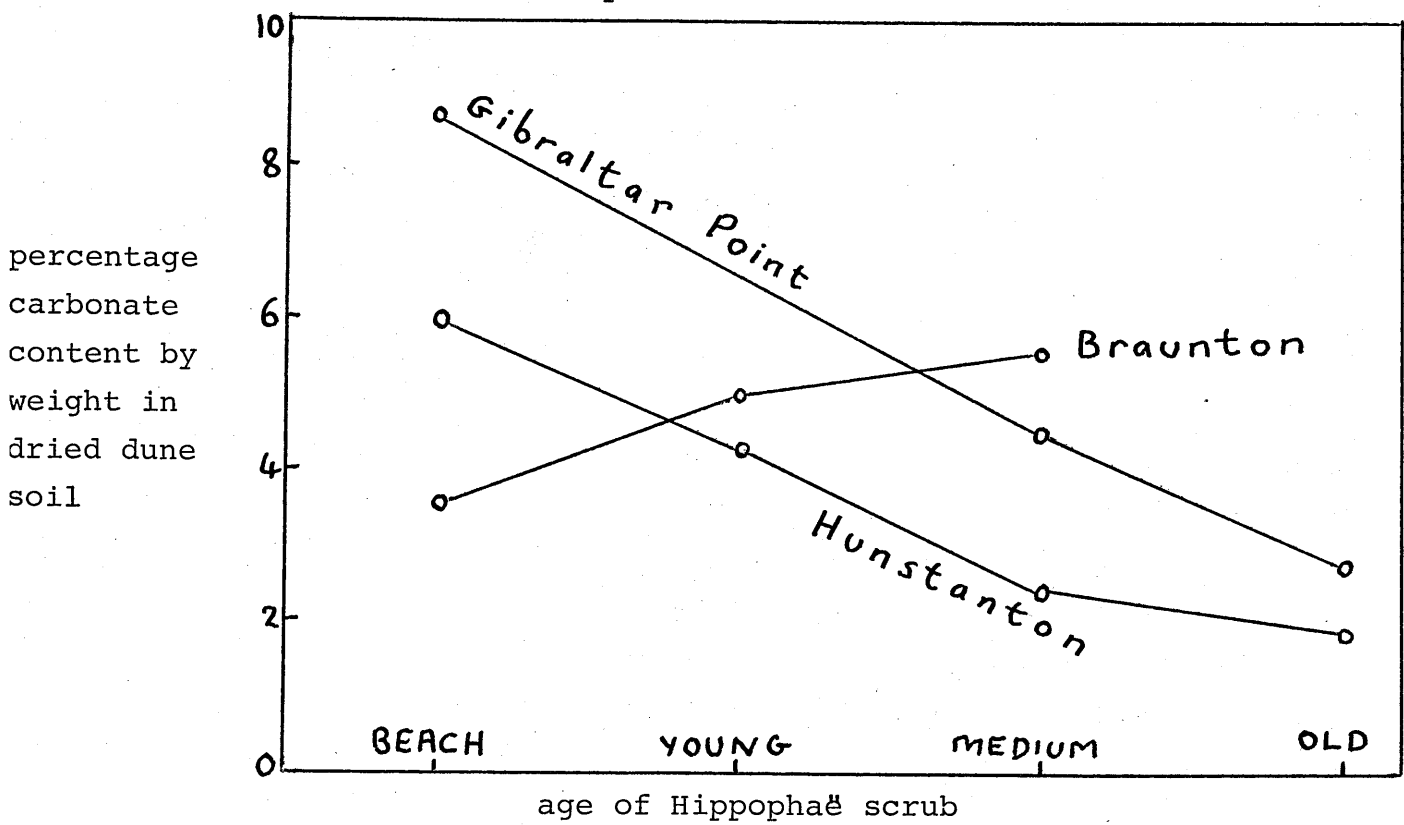


Table 3.7 Percentage weight of carbonate in dune soil  
(mean and standard error Oct. 1981 Hunstanton-  
Holme)

	$\sigma$	$\sigma_n$	max	mean	min
Beach	.4730	.2115	6.72	5.97	5.22
YC	.5225	.2337	4.87	4.26	3.65
YE	.1225	.0548	5.28	5.00	4.70
MC	.4678	.2092	2.96	2.43	1.90
ME	.5404	.2417	2.99	2.32	1.65
OC	.5220	.2335	2.43	1.90	1.37
OE	.6042	.2702	3.64	2.89	2.14

KEY: Y = Young M = Medium O = Old - Sea-Buckthorn  
scrub

C = Control E = Experimental sites

$\sigma$  = standard deviation of the mean

$\sigma_n$  = standard error of the mean

max = maximum error

min = minimum error

t at 5% significance level 4 deg. of freedom = 2.78

Table 3.8 Carbonate content of dune soil at subsidiary  
locations

	Sea Beach	Young Hippo.	Medium Hippo.	Old Hippo.
Gibraltar Point, Lincs.	8.66 9.20 8.24 8.12 9.17	-	2.88 4.45 3.49 6.38 5.22	4.41 3.00 7.13 3.90 6.52
Braunton Burrows, Devon.	2.89 3.62 4.26 2.98 4.15	6.83 5.28 5.09 4.69 3.21	5.77 *	-

The data in the cells are carbonate as a percentage of the dry weight of soil samples.

\* Single sample no replicates

Table 3.9 Statistical analysis of carbonate content of dune soils at subsidiary locations - mean and standard error

Location	Statistic	Sea Beach	Young Hippo.	Medium Hippo.	Old Hippo.
Gibraltar Point, Lincs	maximum	9.31	-	6.21	7.18
	mean	8.68		4.48	4.99
	minimum	8.05		2.75	2.80
Braunton Burrows, Devon	maximum	4.30	6.65	5.77 *	-
	mean	3.58	5.04		
	minimum	2.86	3.43		

difference after some years is not certain. Preventing the downward percolation of organic acids might well reduce the solution and leaching of carbonates but the results in Table 3.6 do not add support to this hypothesis.

The carbonate content of dune soils around the British coasts have been found to vary widely from 75% to less than 1% (CHAPMAN 1964 p. 179). Values given here lie between 2% and 6% which is close to the values of 2-3% quoted by Chapman for Holme, Norfolk. Measurements of soil carbonate were also made at two of the subsidiary sites, Braunton Burrows and Gibraltar Point as indicated in Tables 3.8 and 3.9. The level of carbonate at Gibraltar Point was generally higher than at Hunstanton, but it showed a parallel decline with the advancing age of the scrub. At Braunton Burrows the level of carbonate was somewhat lower tending to the "acid dunes" of the west but the number of samples was really too small for any confidence to be placed in the apparent reversal of the trend. The samples were collected from one large blowout containing sea-buckthorn and there is no proper series of stages as observed at the other two locations shown on Graph 3.12.

### 3.3 Collection of soil samples at depth

In collecting soil samples from beneath sea-buckthorn stands it is important to take them from well below the surface litter, in the region where the root nodules are likely to be present in significant numbers.

A study of sea-buckthorn grown inland (VASCHENKO 1973) indicated the highest frequency roots at a depth of 20 cm, with 80% of roots between 10 and 60 cm. In coastal sea-buckthorn colonies the situation is similar. An analysis of the nodules beneath a stand of sea-buckthorn on the Dutch coast (OREMUS 1979) showed the maximum concentration occurred at 20-40 cm depth, and 80% of nodules were found between 10 and 60 cm depth.

In this study the soil samples were taken from a depth of 20-40 cm, but in the early stages a short survey was made of the variation in a range of constituents with soil depth.

Table 3.10 Soil analysis - Hunstanton

Depth cm	% Water	% Carbonate	% Organic	[N]NO <sub>3</sub>	[N]NH <sub>4</sub> <sup>+</sup>
0	18.9	1.9	6.3	40.2	9.7
10	4.5	2.2	1.0	5.1	0.5
20	2.5	2.1	0.2	1.2	0.5
30	3.1	2.2	0.0	1.1	0.4
40	3.5	2.2	1.1	1.2	0.5
50	3.5	2.5	0.1	1.1	0.6

A half-metre section was excavated beneath a mature (> 12 years) stand of sea-buckthorn. Samples were taken sequentially and analysed for a range of constituents viz:

water content, carbonate content (acid treatment) organic content (loss on ignition) nitrate ion (phenol-disulphonic acid) and ammonium ion (Nessler's reaction). The results are shown in Table 3.10.

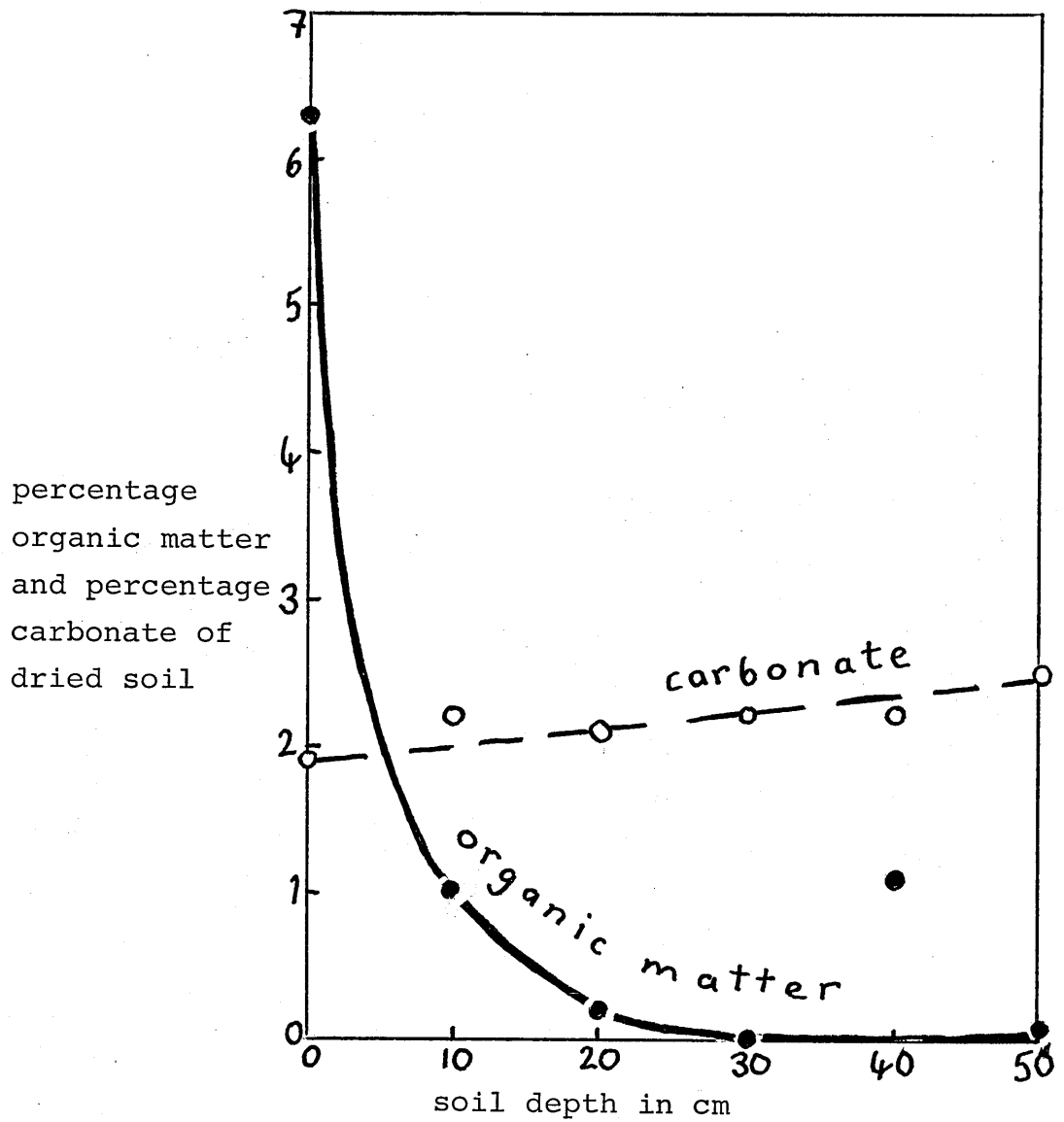
The organic content is high in the litter layer, but it falls off with increasing depth, to about 0.1%. By contrast, the carbonate content is remarkably constant at about 2%.

(Graph 3.13).

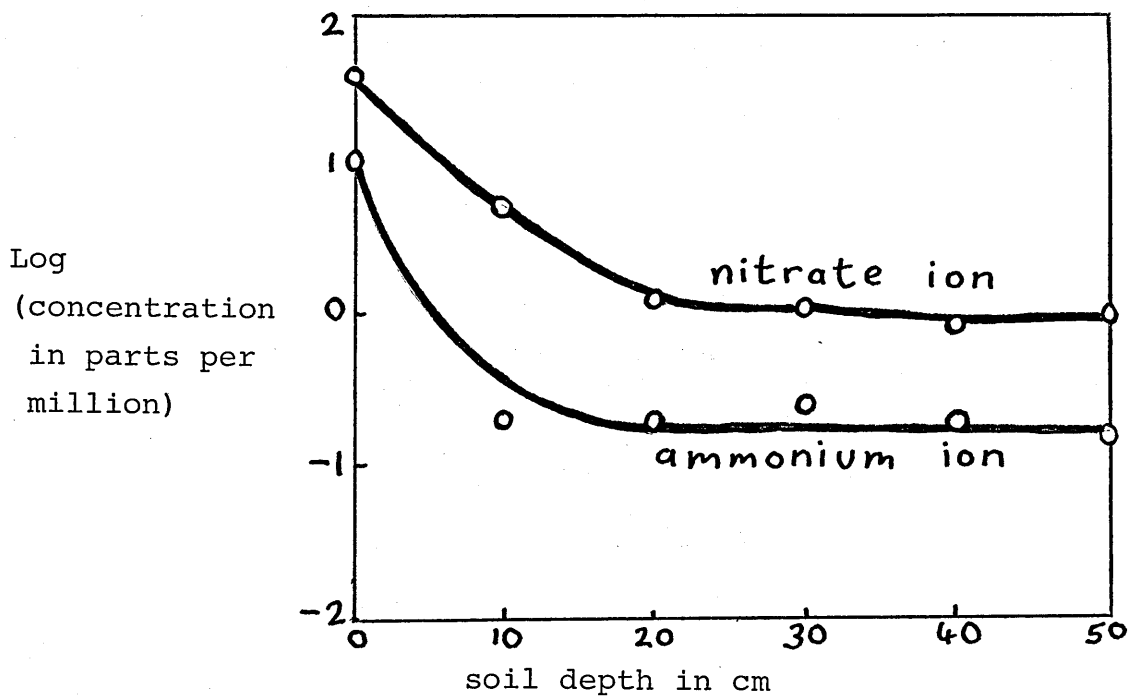
Nitrate and ammonium ion were much higher in the organic litter layer than the deeper soil, where below 10-20 cm their values are constant. The lower value for ammonium ion is probably due to its volatility in comparison with nitrate, and the fact that it is rapidly converted to nitrite and then nitrate. (Graph 3.14).



Graph 3.13 Variation in organic matter and carbonate with depth of dune soil



Graph 3.14 Variation in soluble nitrogen as nitrate and ammonium ions with depth of dune soil



### 3.4 Drying samples of soil

The original soil samples were each split into two lots, a minor bulk, and a major bulk. The latter was used for the main extraction process, and the former was employed to find the water content.

A short control experiment was made to see how much variation might be expected in water content within the same sample of soil, and how much effect this would have on accuracy.

Table 3.11

Six replicates of sample 005	% water content
4.6, 4.8, 5.0, 5.1, 5.0, 4.9	

As Table 3.11 shows the range of variation was from 4.6-5.1, that is 0.5%. This could yield errors in estimation of dry weights, when employing wet weights for extraction. However given other sources of error, this is a very minor difficulty. In measuring nitrate at concentrations of parts per ten million, this problem would give errors of less than one part in a thousand million.

### 3.5 Extraction of soluble materials

Any soluble nitrogen compounds, particularly nitrates derived from breakup of sea-buckthorn nodules can effect the fertility of dune soils and the growth of any additional ground flora. However it is important that these nutrients are freely available to plants. The extraction technique employed de-ionized water, agitation, and a suitable time interval, as a non-destructive method for assessing the kinds of solubles which might reasonably be available to a dune plant.

It was likely that the amount of nitrogenous material present in dune soil was actually greater than that revealed by an aqueous extraction method. The use of potassium chloride in neutral solution (HESSE 1971) was most suitable, given that sandy dune soils have fewer other ions to complicate the process. Hesse recommends that nitrate should be measured as soon as possible to avoid the changes brought about by microbial activity. In this study the delay between collection and extraction was, at most, 48 hours. (HESSE 1971 p. 166, 180).

A parallel set of tests was made. Fifteen soil samples were taken, and each was divided into two. One set of fifteen were subjected to the aqueous extraction, and the other set were extracted by potassium chloride. In the latter case the soil samples were stirred with 1 M potassium chloride for one hour, and the lixiviate was filtered with glass wool, and "postlip" papers in a Büchner funnel. The filtrate was diluted to a given volume, aliquots taken, and the excess chloride precipitated with saturated silver sulphate solution.

After further filtration, the solutions were evaporated and the nitrate level estimated by the standard phenol-disulphonic acid method.

The results of the parallel tests are given in Table 3.12.

Table 3.12 Comparison of two methods of extracting solubles from soil samples

Status	Sample	Cl[N]	Aq[N]
Beach	261	1.48	0.20
	262	0.72	0.09
	263	1.53	0.17
	264	1.09	0.14
	265	1.29	0.25
$O_C$	286	0.34	0.30
	287	1.98	0.63
	288	4.87	1.74
	289	0.96	1.43
	290	3.07	0.47
$O_E$	291	5.69	1.30
	292	8.89	0.57
	293	3.91	1.02
	294	5.91	4.41
	295	5.04	1.00

Cl[N] = concentration of nitrogen in ppm dry soil - chloride extracted.

Aq[N] = concentration of nitrogen in ppm dry soil - aqueous extracted.

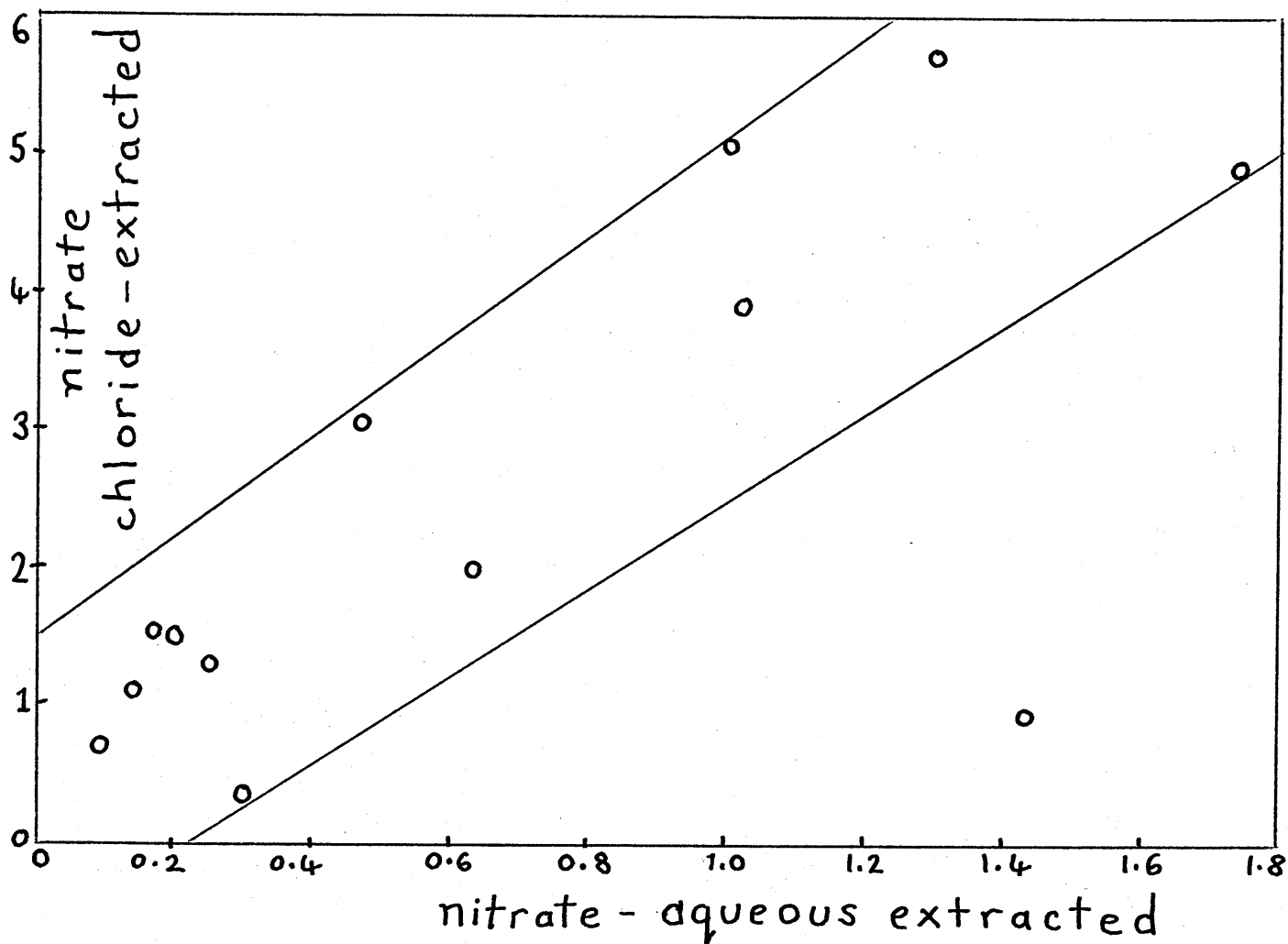
$O_C$  = old scrub - control sites

$O_E$  = old scrub - exptal sites

The results show considerable variation. In all but one sample the chloride-extracted values are higher, as had been expected. The general trend and extent of variation is shown in Graph 3.15 and the regression line indicates that  $x = 3.57 y$  where  $x = Aq[N]$  and  $y = Cl[N]$ .

There is much value in making Kjeldahl estimations of total nitrogen, but given the facilities available it was not practicable here. In heavier soils the real extent of soil

Graph 3.15 Comparison of nitrate ion recorded from dune soil by aqueous and chloride extraction.



Key - nitrate, chloride-extracted = concentration of nitrogen as nitrate ion, extracted by potassium chloride solution

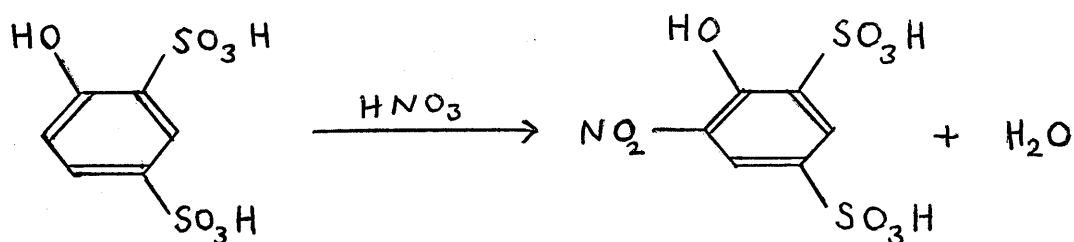
nitrate, aqueous extracted = concentration of nitrogen as nitrate ion, extracted by de-ionized water.

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nitrogen is often concealed by the action of clay mineral constituents which strongly adsorb some inorganic ions. Kjeldahl digestion methods help to break down the adsorbed nitrogen compounds for assay (BREMNER 1960). However poor dune soils are relatively free of clay minerals, and the need for Kjeldahl methods is much less critical.

### 3.6 Analysis for nitrate ion

The procedure followed for the determination of nitrate ion quantitatively is based on the nitration of phenol-disulphonic acid, first described in 1864 by Sprengels, and subject to continuing modifications. The nitrate present in the sample must be perfectly dry as water interferes with the reaction. Excess of phenol-disulphonic acid is employed, and the extent of nitration is dependent upon the amount of nitrate ion present in the sample.



The product of the reaction is colourless in acid solution, but when made alkaline with ammonia it forms a bright yellow tri-ammonium salt whose colour is directly proportional to the amount of nitrate taken up. (HESSE 1971 p. 181).

In the pilot study nitrate was estimated colorimetrically by the use of a BDH Lovibond comparator which employs a series of standard colour discs corresponding to concentrations of 2.5 to 30 mg of N as  $\text{NO}_3^-$  per 50 ml solution. The main source of error here is that the matching process is a subjective one, and the presence of haze creates considerable problems. With a path length of 100 mm the haze effect is magnified.

Nevertheless the results obtained initially are very comparable with those achieved later by spectrophotometer.

All the results in the main study were obtained by determining the absorbance of the test solutions of nitrated phenol-disulphonic acid (NPDSA) in comparison with standard nitrate solutions.

The problem of haze was largely eliminated by filtering the soluble extract through precipitated chalk. With a path-length of 10 mm this source of error was negligible.

A series of 10 ml aliquots from the known masses of soluble extract was pipetted into 40 ml glass vials. These solutions were evaporated to dryness. It was critical that the samples were heated for long enough to drive off all water, but not so long that the organic components of the residue were charred. The brown colour produced by charring was examined as a spectrophotometric trace and it was found to absorb in the yellow wavelength used for nitrate determination.

The dried residue was covered with a solution of 25% w/v phenol-disulphonic acid in concentrated sulphuric acid. A burette was used to measure 1 ml of the solution for each vial, to ensure that there was an excess, but not so much as to require large volumes of ammonia for neutralization. Each vial was turned and tilted to ensure the PDSA solution made contact with all of the residue, and the reaction was timed so that 15 minutes, at least, was available for nitration.

The acid solution was diluted with 10 ml of de-ionized water and cooled. Finally the vials were made up to 30 ml with concentrated .880 ammonia which developed the yellow colour



64v  
of NPDSA triammonium salt. This is stable for at least a week, but in most cases the spectrophotometric examination took place on the day following the nitration reactions.

Along with the test solutions, a series of 10 ml samples of NPDSA of known strength were prepared. These standards had been made by taking aliquots of very dilute ammonium nitrate solutions and treating them in the same way as the test solutions. Given the identical treatment of test solutions and standards it was only necessary to relate absorbences to find the concentrations of the unknown solutions. The standards normally employed were 200, 100, and 50  $\mu\text{M}$  of N as nitrate ion. A graphical plot of absorbence against concentration was made for each batch of standards. If the instrument response was linear it was possible to proceed with determining the absorbencies of test solutions.

It has been recommended that fresh standards are always employed for each batch (HESSE 1971 p. 183) and this proved valuable for checking back on results. On most batch runs absorbence was measured at 420 nm on a Pye Unicam SP8-100 spectrophotometer, but a Perkin-Elmer-Coleman 575 instrument was also used on occasions. Hesse suggests that transmission should be measured at 410 nm. This is in the blue-violet end of the spectrum where NPDSA transmits little light, so absorbence seems a more appropriate parameter to measure. There is only one characteristic absorbence peak for NPDSA, and this has a plateau between 410 and 420 nm.

A pair of matched, 1 cm path-length quartz cuvettes were used. The reference solution consisted of 1 ml of PDSA made

up to 30 ml with ammonia. There was a minimal difference between using this or plain ammonia as a reference standard. The instrument was adjusted to zero with the reference solution in both the reference and test cuvettes. During the course of examining the test solutions about every tenth determination the reference standard was used in the test cuvette to see if there had been drift on the instrument so that re-adjustment could be made.

As a check on contamination of solutions, a couple of blanks were used. When preparing standards, and test solutions two vials were made up with 10 ml of de-ionized water evaporated to dryness and then treated in the same way as the rest. In no case did the blank solutions ever yield an absorbance reading. This indicates that the very low nitrate concentrations measured were not due to contamination of the vials, or reagents at any stage of the analytical programme.

It is known that chloride ion in concentrations above 10  $\mu\text{g}$  per ml in the test solution will interfere with the PDSA reaction and must be removed. (HESSE 1971 p. 181). Some of the early test solutions were assayed for presence of chloride ion. This proved to be sufficiently low to be unlikely to interfere with the PDSA reaction. (Table 3.13).

Table 3.13 Level of chloride ion in samples

Sample	$W_d$	S	V	$\text{Cl}^-$ -M	$\text{Cl}^-$ $\mu\text{M}$	Wt. $\text{Cl}^-$	[Cl]ppm
001	31.64	100	0.45	.000167	167.4	5.9	18.5
002	35.10	100	0.25	.000093	93.0	3.3	9.3
008	36.79	100	0.60	.000223	223.2	7.9	21.2

Key for Table 3.13:

$W_d$  = dry weight of soil in g

$S$  = volume of soluble extract - ml

$V$  = volume of 0.0093  $\text{AgNO}_3$  = 25 ml soluble extract

$\text{Cl}^- \text{M}$  = molarity of soluble extract chloride

$\text{Cl}^- \mu\text{M}$  = micromolarity of soluble extract chloride

$\text{Wt. Cl}^-$  = weight of chloride as  $\mu\text{g}$  per ml

$[\text{Cl}] \text{ppm}$  = concentration of chloride in ppm dry soil

$$\text{Cl}^- \text{M} = \frac{0.0093V}{25} \qquad \text{Cl}^- \mu\text{M} = \frac{\text{Cl}^- \text{M}}{10^6}$$

$$\text{WtCl}^- = \text{Cl}^- \mu\text{M} \times \frac{35.5}{10^3}$$

$$[\text{Cl}] \text{ppm} = \frac{35.5}{10^3} \times \frac{\text{Cl}^- \mu\text{M} S}{W_d}$$

Given the precautions outlined above, the accuracy of this method of estimating nitrate concentration is subject to a 5% error (HESSE 1971 p. 182).

### 3.7 Analysis for ammonium ion

The level of ammonium ion in soil extracts was measured, but these results do not form a major part of the study. There are three main reasons; the level of ammonium ion was always very small; it is difficult to measure low concentrations with any accuracy; and there does not appear to be any relationship between ammonium levels and the age of the buckthorn scrub. Much of this part of the investigation was an attempt to discover and eliminate sources of error.

Initially ammonium ion was estimated by a technique involving Nessler's reaction. The reagent is a strongly alkaline solution of sodium hydroxide, containing mercury II chloride in potassium iodide (SNELL & SNELL 1949). The reaction with ammonium ion produces a colloidal orange-brown solution, with a strong tendency to precipitate. The yellow colour of very dilute solutions is proportional <sup>to</sup> the concentration of ammonium ion, and the values of test solutions were estimated using a series of standard colour discs (1-100  $\mu\text{g N as NH}_4^+$  per 50 ml) within a BDH Lovibond comparator.

Given the 100 mm vertical optical path length, the effect of precipitation was marginal and an experiment with a range of standard ammonium sulphate solutions showed that the comparator gave quite accurate results (Table 3.14).

Table 3.14 Comparator assay of standard solutions  
 $\mu\text{g Nas NH}_4^+$  per 50 ml

N standard	86	71	63	57	34	28	23	27	6
N estimated	80	70	60	56	34	38	23	15	4

The method, however, suffers from the same drawbacks as the use of comparator discs gave for nitrate determination,

notably the subjective nature of the comparison, and problems of haze in a wide-spectrum examination.

When the spectrophotometer was used to examine standard solutions further difficulties emerged. Unless the solution was extremely dilute, it absorbed very strongly over much of the visible wavelength range, and exceeded the instrumental capacity. Even with dilute solutions, the reaction could be observed to proceed continuously, gradually increasing the absorbence value. It was necessary to measure absorbence at a fixed time from the start of the Nessler reaction, viz two minutes. The problem of carrying out manipulations, and reactions immediately prior to absorbence measurement was not conducive to accuracy.

The results obtained from an assay of 30 soil sample extracts is given in Table 3.15. The absorbencies were measured at 400 nm and appear to be quite satisfactory notwithstanding the fact that no variation in ammonium level with the age of the scrub is evident. (Graph 3.16).

However, a more sensitive and controlled assay technique revealed a consistent error hidden by the Nessler reaction. This is the method of indophenol formation with sodium salicylate (VERDOUW 1978).

One great advantage is that the reactions on the test and standard solutions can be carried out independently of the spectrophotometer measurements. The blue indophenol compound, once it is properly developed by bright light, is stable for at least a year, according to Verdouw's

experiments. The solution is clear, and free of precipitates with strong absorbence peaks, and very low concentrations of ammonium ion produce distinct blue colours, indicating that it is a very sensitive test.

Table 3.15 Ammonium ion concentrations assayed by indophenol method

data in cells = N as  $\text{NH}_4^+$  in ppm dry soil

Age of Scrub	Control Replicates	Experimental Replicates
young	0.2, 0.3, 0.3, 0.3, 0.4	0.2, 2.3, 1.7, 2.0, 3.0
medium	0.5, 0.4, 0.3, 0.3, 0.2	1.3, 0.3, 0.3, 0.3, 0.2
old	0.7, 0.3, 0.3, 0.2, 0.2	0.4, 0.3, 0.2, 0.2, 1.1

data in cells = mean and standard error

		Age of Scrub	maximum	mean	minimum	on t
Treatments	Control	young	0.39	0.30	0.21	0.09
		medium	0.48	0.34	0.20	0.14
		old	0.60	0.34	0.08	0.26
	Exptal	young	3.13	1.84	0.55	1.29
		medium	1.05	0.48	0.00	0.57
		old	0.91	0.44	0.00	0.47

This assay technique simply involves mixing 10 ml of the water under test, with three other reagents, and leaving the mixture for 2 hours in bright light (overhead fluorescent lighting) for the colour to develop. Verdouw's solution specifications were slightly modified in the following way.

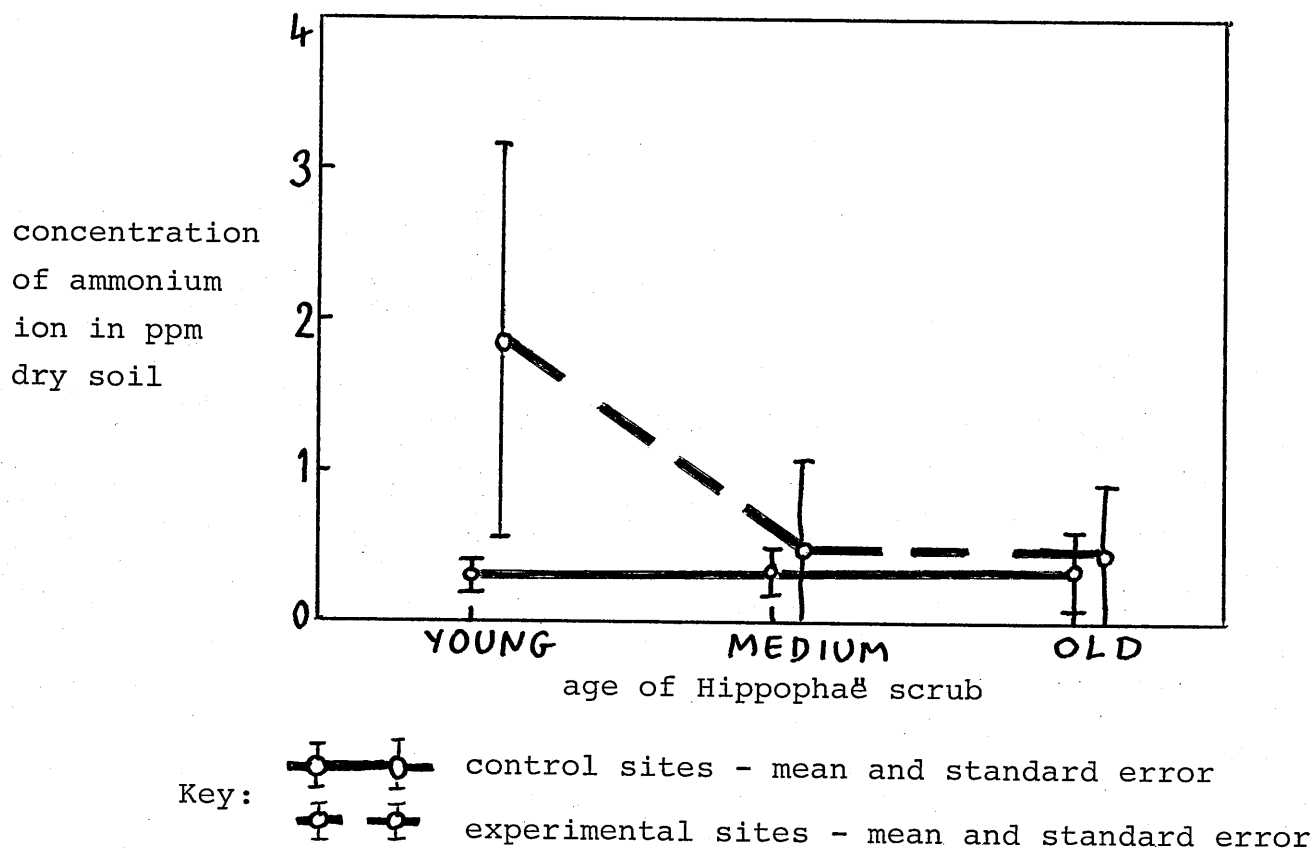
Test water - 10 ml

A solution - 5 ml (40% sodium salicylate)

B solution - 2 ml (5% sodium hypochlorite in 0.1 NaOH)

C solution - 5 ml (2% potassium ferrocyanide, 10% sodium citrate in 0.1 N NaOH)

Graph 3.16 Variation in levels of ammonium ion in dune soil with age of Hippophaë scrub (data from Hunstanton)



The reference sample was de-ionized water treated in the same way as the standards, and test solutions. The absorbences were measured at 660 nm.

On the first occasion the reference solution proved to be absorbing more strongly than half of the test solutions, and this invalidated the results. Redistilled de-ionized water is recommended (VOGEL 1962) as a suitable ammonia-free water necessary for all ammonia assay methods.

Further attempts to obtain a satisfactory ammonia-free water were unsuccessful. Reference solutions, and blanks on each occasion showed a pale blue colour indicating the presence of ammonia. Given the need for quantities of concentrated ammonia in the nitrate assay, it was impossible to keep the laboratory atmosphere free of ammonia, and consequently the entire range of assays of ammonium ion in soil extracts must be considered suspect.



### 3.8 Analysis for phosphate ion

It was hoped that estimation of phosphate in dune soil extracts might provide evidence for the possible contribution of animal excretions to the dune soil economy. The method used was that proposed by Denigès, and modified later by Holman and Pollard (1937). It involves the reduction of dodeca-molybdophosphoric acid with fresh stannous chloride solution to produce a deep blue colour. In low concentrations, this colour is proportional to the level of orthophosphate present in the test solution.

The reaction is free from interference by most of the commonly occurring ions, except iron (III). It is very sensitive to low concentrations of orthophosphate, and the blue solution is stable for several days.

It is necessary to reduce iron(III) when present in concentrations above 1 ppm. In this case a Jones reductor - freshly prepared zinc-mercury amalgam was used. When the concentration of phosphate is above about 0.4  $\mu\text{g}$  per ml it tends to produce too strong a blue colour and the absorbance begins to depart from the Lambert-Beer Law. (HESSE 1971 p. 291).

Organic acids may also interfere, but in soils as poor as those of the young dunes the levels were likely to be extremely low.

Soluble extracts from the soil samples were filtered through precipitated chalk in a Büchner funnel and then passed through a 50 cm column packed with zinc-mercury amalgam on glass fibre.

The 10 ml aliquots of the test solutions were each mixed with 1 ml ammonium molybdate reagent and 0.15 ml fresh stannous chloride solution, and made up to 50 ml with distilled water. The reference solution was a 10 ml sample of de-ionized water treated in the same way as the test solutions. Standards with concentrations of 20, 50, 100, and 200  $\mu\text{M}$  phosphate were also prepared. The samples were examined in a Pye Unicam SP8-100 spectrophotometer at a wavelength of 700 nm to measure absorbence.

The results of the early analysis gave very low, but consistent levels of phosphate in the dune soil (Table 3.16). The usual range was from about 0.1 to 0.3 ppm of P in dry soil. Just two out of the thirty samples showed higher values (0.6 and 0.8). The graph of phosphate level against scrub age seems to indicate slightly higher levels in the medium scrub (Graph 3.17).

A much later analysis of dune soil samples (Table 3.16) where a marked contrast between control and experimental sites might be expected, yields very low results for the presence of phosphate ion. These range from zero to 0.09 ppm dry soil, with one exceptional result at 0.56 ppm.

Given these extremely low levels it seems unwise to place any reliance upon any differences observed between controls and experimental sites, not because the results are necessarily inaccurate, but given the sources of error inherent in the experimental procedures, the differences at these low levels are likely to be the product of pure chance. In Graph 3.17 it is clear that the standard error is large and sufficient to cast doubt on the trend of the means.

Table 3.16 Phosphate analysis - Hunstanton

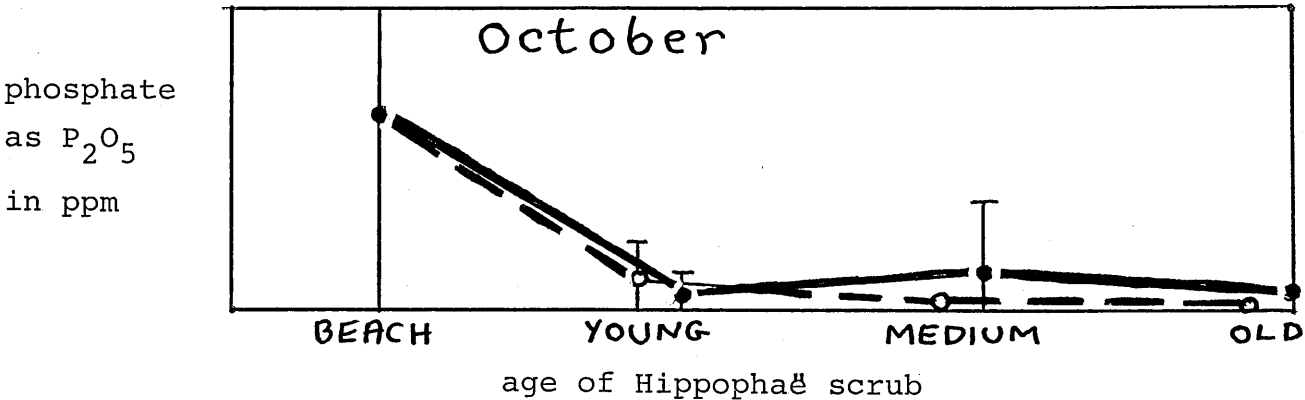
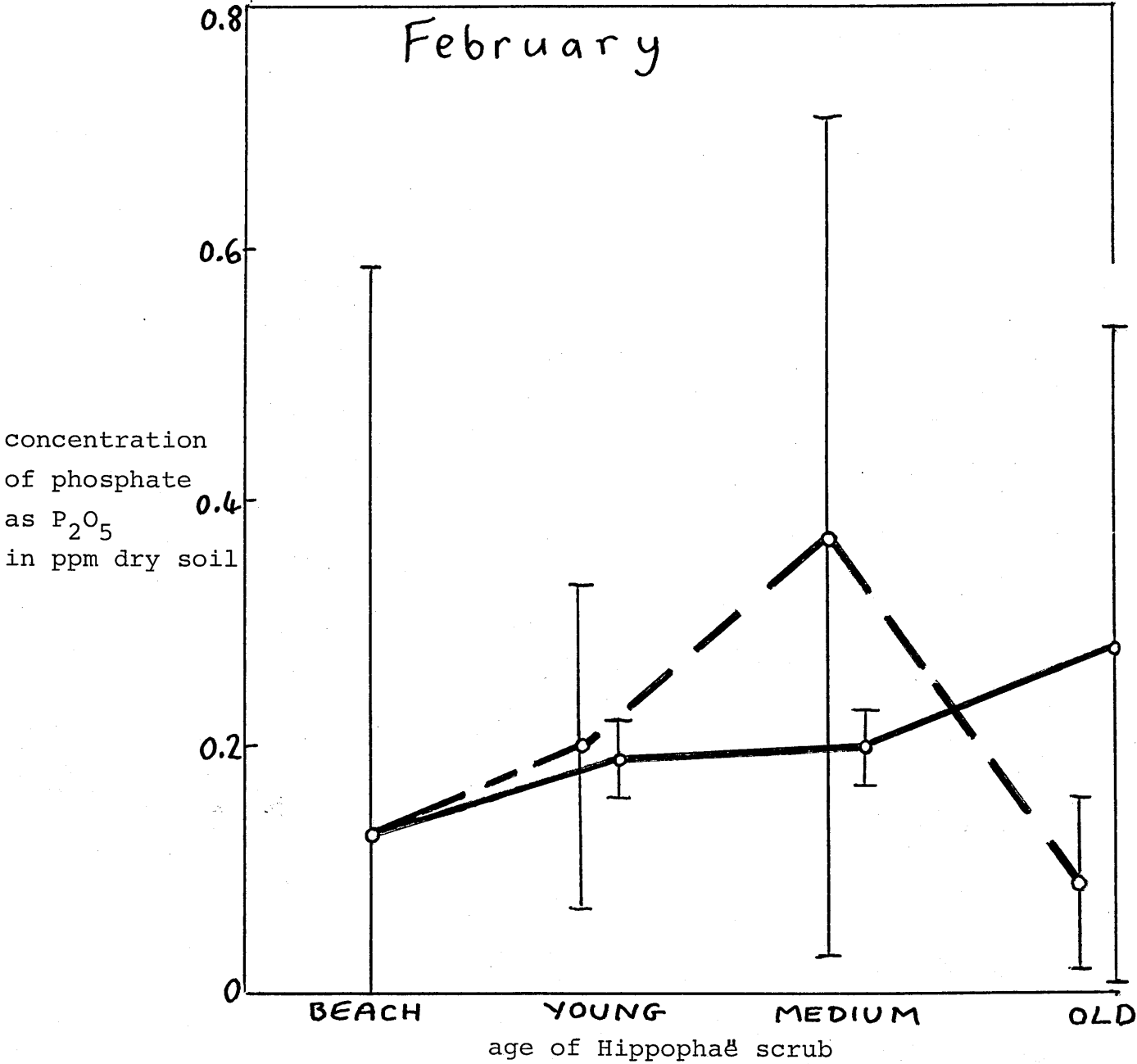
Age	February		October	
	Control	Exptal	Control	Exptal
Young	0.17	0.22	0.03	0.03
	0.19	0.13	0.01	-
	0.18	0.15	0.00	0.02
	0.24	0.14	0.01	0.03
	0.19	0.38	0.00	0.00
Medium	0.19	0.84	0.09	0.01
	0.22	0.28	0.02	0.01
	0.21	0.24	0.00	0.01
	0.17	0.32	0.01	0.00
	0.23	0.16	0.01	0.00
Old	0.12	0.09	0.01	0.01
	0.25	0.12	0.01	0.00
	0.20	0.00	0.01	0.00
	0.67	0.08	0.01	0.00
	0.18	0.16	0.01	0.00
The data here are phosphate concentrations as ppm of phosphate as $P_2O_5$ .			Beach	0.56
				0.04
				0.02
				0.02
				0.03

Perhaps the most curious aspect of these results is the striking difference between the means for February and October. The former are larger by an order of magnitude. It is possible that this is a reflection of experimental technique, as the first assay (of the February samples) was made on 20.5.81, and the second assay (of October samples) was made on 3.12.81. Laboratory notes indicate problems with the colorimetric reaction and the spectrophotometry.

Given these low levels of soluble phosphate it seems unlikely that animal excretions play much part in the nitrogen enhancement of dune soils at the early stages in their development. The absence of, and clear difference between controls and experimentals tends to confirm this. However the whole issue of phosphate chemistry in dune soils seems to be more complicated than had been anticipated.

Graph 3.17    Variation in levels of soluble phosphate ion in dune soil with age of Hippophaë scrub (data from Hunstanton)

Key: ●—● controls                      mean and standard error  
○—○ experimentals                    mean and standard error



Chapter 4: Analysis of Results  
Discussion and Conclusions

#### 4.1 Principal project - Graphical analysis

The results from the principal project at Hunstanton are presented in Table 4.1 and consist of raw data for nitrate levels in the dune soil, showing the extent of seasonal variations. Four blanks appear in the table as a label was lost from an experimental site after the August collection, and a soil sample was lost in February.

Mean and Standard error of the replicates for each site was calculated as indicated in Section 4.2, for 5% confidence limits. As the number of replicates is small the range of these limits tends to be rather large in some cases.

Occasionally the deviation was large enough to reduce the lower limit to zero. The range of these confidence limits is given in Table 4.2 and then displayed in Graph 4.1, but omitted from subsequent graphs in the interests of clarity.

##### 4.1.1 Seasonal Variation in soil nitrate level

The pattern of seasonal variation is shown in Graphs 4.2 and 4.3. These indicate that the nitrate levels in soils under older stands of *Hippophaë* are markedly greater in April compared with the rest of the year, in both control and experimental sites. A somewhat smaller peak occurs in the results for September. The results for medium, and young stands follows this general picture, although the medium experimental sites showed a fall in September in contrast with the general rise. It is not easy to see why the two peaks should occur at those particular times of the year.

Table 4.1 Seasonal variation in nitrate levels in dune soil  
 - Hunstanton - raw data - nitrate as ppm dry soil

1. Control Sites

Age	Feb	April	June	Aug	Sept	Oct	Nov
Young	0.15	0.22	0.05	0.40	0.72	0.13	0.15
	0.13	0.27	0.03	0.31	0.34	0.15	0.21
	0.11	0.23	0.08	0.48	0.40	0.20	0.09
	0.10	0.21	0.28	0.30	0.66	0.13	0.11
	0.10	0.20	0.04	0.30	0.22	0.07	0.14
Medium	0.19	0.58	0.37	0.36	0.24	0.18	0.15
	0.37	0.45	0.10	0.45	1.17	0.11	0.44
	0.22	1.40	0.04	0.20	0.06	0.14	0.11
	0.30	0.08	0.09	0.23	0.16	0.15	0.19
	0.38	2.19	0.58	0.29	0.21	0.14	0.31
Old	0.17	3.26	0.40	0.54	0.87	0.33	0.30
	0.12	3.74	0.33	0.76	1.39	0.42	0.63
	0.42	3.54	0.15	0.66	1.90	0.47	1.74
	0.22	0.18	0.34	0.86	1.27	0.40	1.43
	0.26	5.67	1.00	1.14	1.08	0.68	0.47

2. Experimental Sites

Age	Feb	April	June	Aug	Sept	Oct	Nov
Young	0.45	0.32	0.00	0.11	0.23	0.11	0.22
	0.18	0.41	0.05	0.56	-	-	-
	0.10	0.24	0.00	0.47	0.73	0.21	0.08
	0.13	0.23	0.00	0.87	0.68	0.25	0.10
	0.09	0.22	0.08	0.78	0.57	0.16	0.20
Medium	0.13	1.27	0.15	0.72	0.41	0.27	0.16
	0.43	0.22	0.24	0.75	0.58	0.26	0.18
	-	1.36	0.47	1.61	0.37	0.23	0.44
	0.18	1.90	0.24	0.56	0.41	0.17	0.25
	1.36	1.04	0.26	1.29	0.52	0.20	0.50
Old	0.11	0.85	0.31	1.33	1.78	0.28	1.30
	0.30	0.93	0.32	0.60	2.61	0.45	0.57
	0.21	4.71	2.25	1.41	5.67	0.42	1.02
	0.46	3.45	1.76	0.75	2.99	0.45	4.41
	0.24	3.24	0.85	1.87	2.20	0.85	1.00

Table 4.2 Mean seasonal variation in nitrate levels in dune soil - Hunstanton (mean and standard error within 5% confidence limits)

1. Control Sites

Age	Statistic	Feb	April	June	Aug	Sept	Oct	Nov
Young	$\Sigma x/n$	0.12	0.23	0.10	0.36	0.47	0.14	0.14
	$\sigma$	0.022	0.027	0.105	0.080	0.214	0.047	0.046
	$\sigma n$	0.010	0.012	0.047	0.036	0.096	0.021	0.021
	5% Dev	0.03	0.03	0.13	0.10	0.27	0.06	0.06
	maximum	0.15	0.26	0.33	0.46	0.74	0.20	0.20
	minimum	0.09	0.20	0.00	0.26	0.20	0.08	0.08
Medium	$\Sigma x/n$	0.29	0.94	0.24	0.31	0.37	0.14	0.24
	$\sigma$	0.086	0.849	0.232	0.101	0.454	0.203	0.135
	$\sigma n$	0.038	0.380	0.104	0.045	0.203	0.011	0.060
	5% Dev	0.11	1.06	0.29	0.13	0.56	0.03	0.17
	maximum	0.40	2.00	0.53	0.44	0.93	0.17	0.41
	minimum	0.18	0.00	0.00	0.18	0.00	0.11	0.07
Old	$\Sigma x/n$	0.24	3.28	0.44	0.79	1.30	0.46	0.91
	$\sigma$	0.115	1.975	0.325	0.228	0.388	0.133	0.633
	$\sigma n$	0.051	0.883	0.145	0.102	0.174	0.059	0.283
	5% Dev	0.14	2.46	0.40	0.28	0.48	0.17	0.79
	maximum	0.38	5.74	0.84	1.07	1.78	0.63	1.70
	minimum	0.10	0.82	0.04	0.51	0.82	0.29	0.12

mean =  $\Sigma x/n$

standard deviation =  $\sigma$

standard error =  $\sigma n$

deviation within 5% confidence limits = 5% Dev

maximum limit

minimum limit



## 2. Experimental Sites

Age	Statistic	Feb	April	June	Aug	Sept	Oct	Nov
Young	$\Sigma x/n$	0.190	0.284	0.026	0.558	0.553	0.183	0.150
	$\sigma$	0.150	0.081	0.037	0.298	0.225	0.061	0.070
	$\sigma n$	0.067	0.036	0.017	0.133	0.113	0.030	0.035
	5% Dev	0.19	0.10	0.05	0.37	0.36	0.10	0.11
	maximum	0.38	0.38	0.08	0.93	0.91	0.28	0.26
	minimum	0.00	0.18	0.00	0.19	0.19	0.08	0.04
Medium	$\Sigma x/n$	0.525	1.158	0.272	0.986	0.458	0.226	0.306
	$\sigma$	0.572	0.612	0.119	0.444	0.088	0.042	0.155
	$\sigma n$	0.286	0.274	0.053	0.199	0.039	0.019	0.069
	5% Dev	0.91	0.76	0.15	0.55	0.11	0.05	0.19
	maximum	1.44	1.92	0.42	1.54	0.57	0.28	0.50
	minimum	0.00	0.40	0.12	0.44	0.35	0.18	0.12
Old	$\Sigma x/n$	0.264	2.636	1.098	1.192	3.050	0.490	1.660
	$\sigma$	0.129	1.690	0.874	0.518	1.533	0.213	1.559
	$\sigma n$	0.058	0.756	0.391	0.232	0.685	0.095	0.697
	5% Dev	0.16	2.10	1.09	0.64	1.91	0.26	1.94
	maximum	0.42	4.74	2.19	1.83	4.96	0.75	3.60
	minimum	0.10	0.54	0.01	0.55	1.14	0.23	0.00

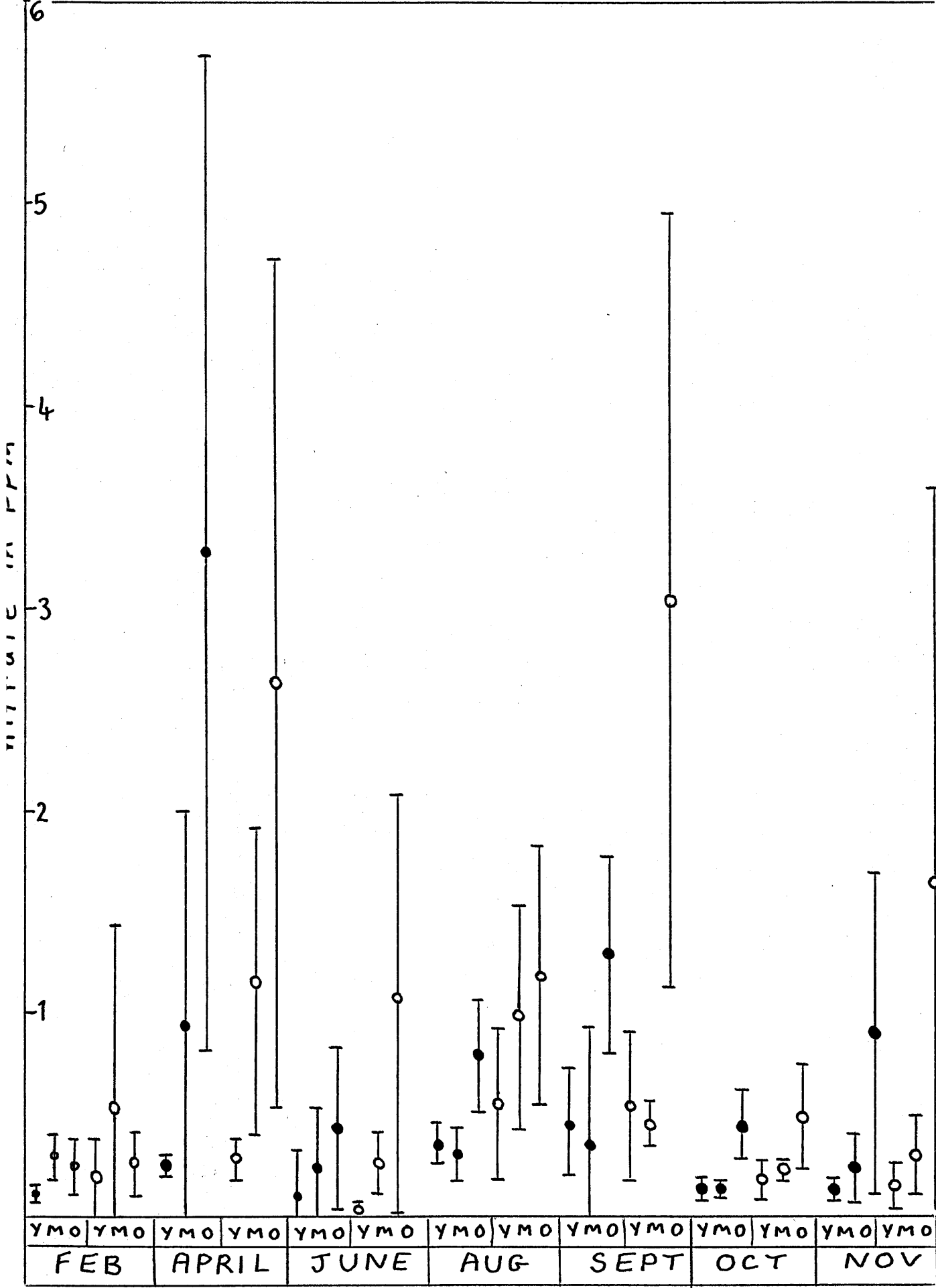
There are probably three processes going on within the dune soil; (i) the bacterial decay of root nodules of *Hippophaë* leading to nitrate production; (ii) the denitrification of nitrate in the soil and its conversion to dinitrogen by bacteria such as *Pseudomonas* and *Achromobacter*; and (iii) the uptake of nitrate by green plants growing in the soil.

It is likely that (i) and (ii) are temperature dependent and tend to reach a maximum in the warmer months between about April and October. By contrast, process (iii) is dependent upon the much slower growth of dune plants reach a maximum about June, and declining after flowering, in September. Many

Graph 4.1

Levels of soluble nitrate during the year in parts per million of dried dune soil; - means and 5% confidence limits.

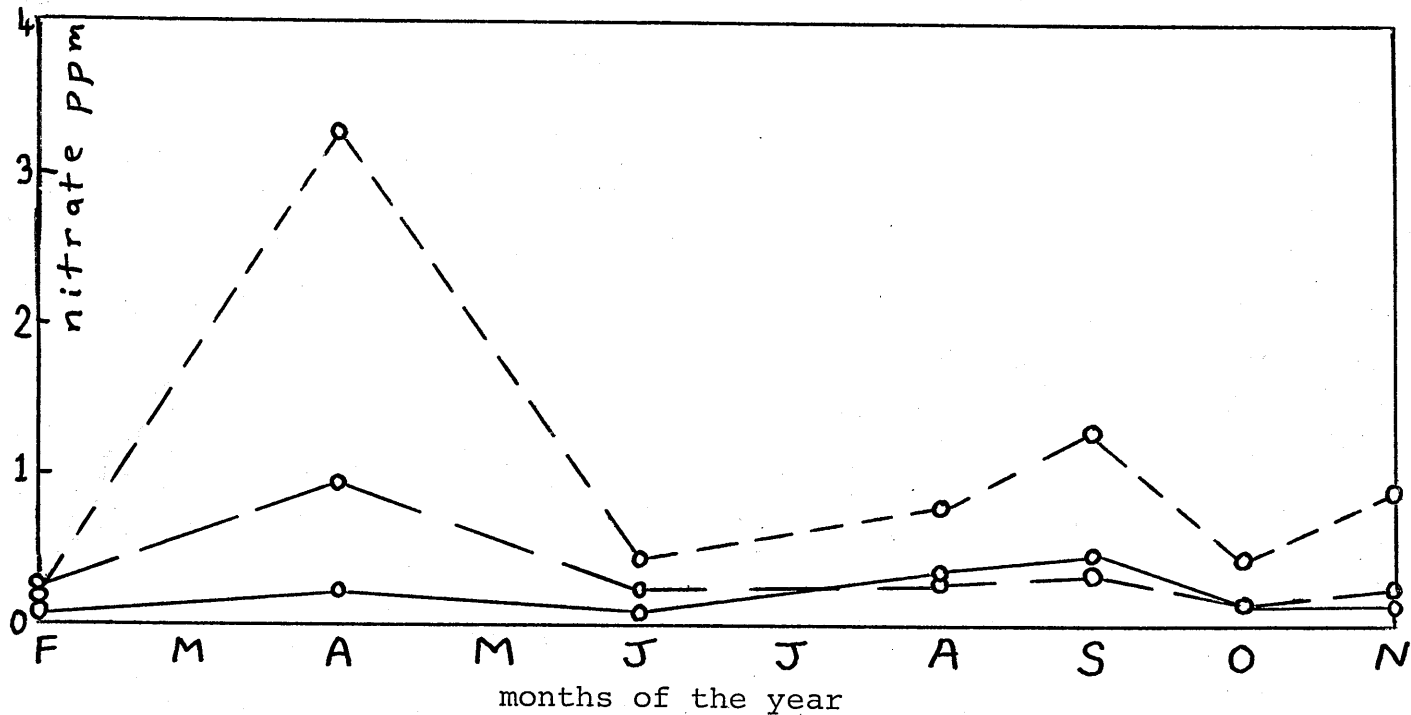
Key: ● control sites      ○ experimental sites



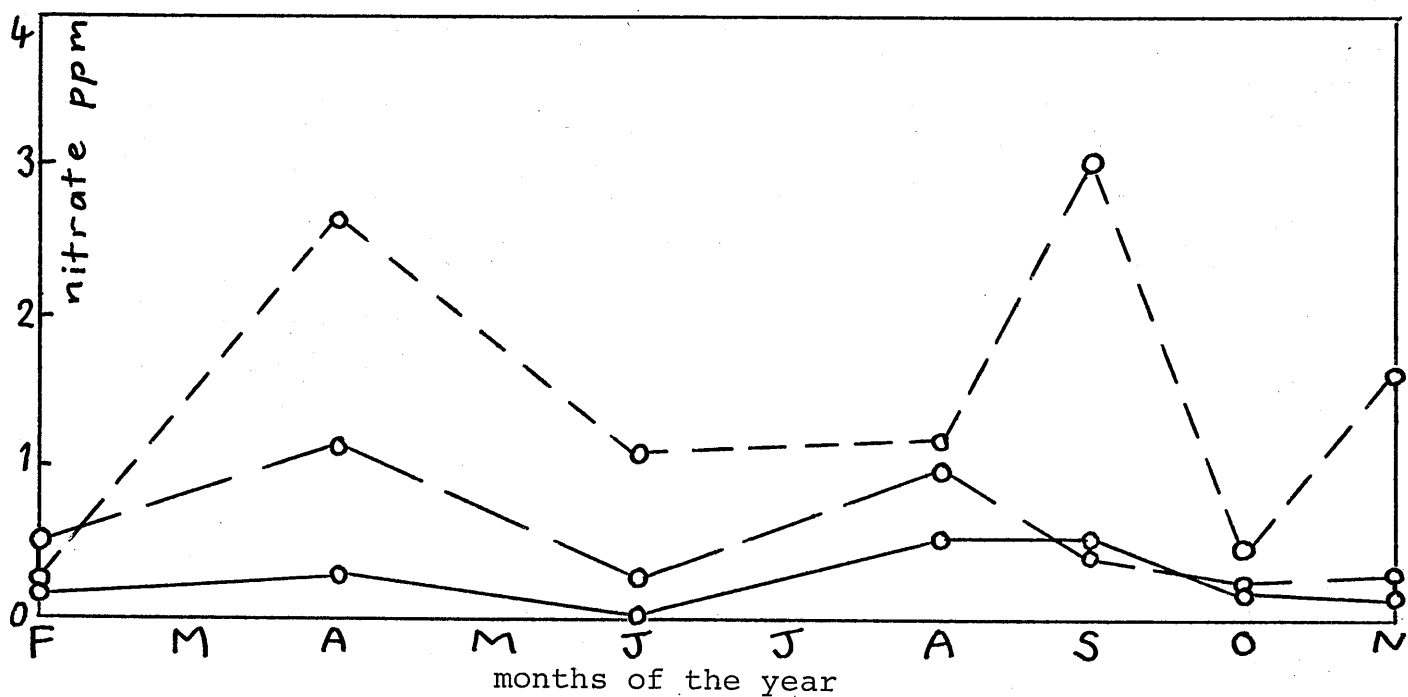
Seasonal variations in levels of soluble nitrate in dune soils  
(data from Hunstanton)

Key:   
 ○ --- ○ old scrub   
 ○ — ○ medium scrub   
 ○ — ○ young scrub

Graph 4.2 Control sites



Graph 4.3 Experimental sites



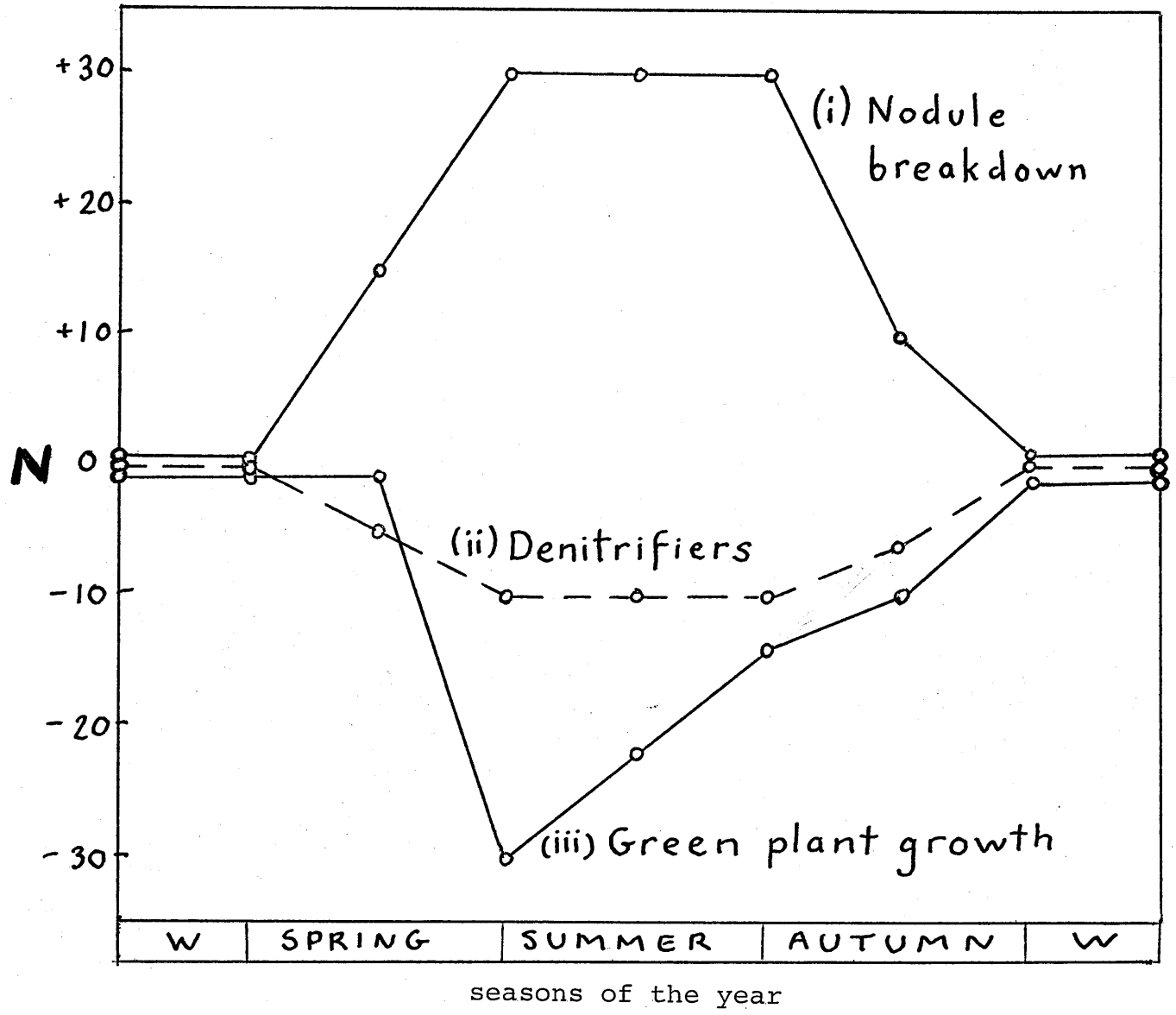
of these plants are annuals, like *Senecio jacobaea* or they have a relatively short period of green leaf growth followed by die-back, like *Ammophila arenaria*.

Figure 4.1 is a simple model of the nitrate economy of a dune soil produced by quantifying the three propositions in the preceding paragraphs. In A the two bacterial populations (i) and (ii) are shown to have symmetrical physiological responses about the midsummer temperature maximum. The plant growth graph (iii) shows a rapid uptake of nitrate in the spring and early summer followed by a slow decline on maturity.

In B the seasonal variation in soil nitrate level is the sum of the three processes in A, superimposed on a winter base level, to which the graph returns at the end of the summer. Of course it is one thing to describe a model which fits the observed variations and quite another to be able to prove the details. This would require a very carefully designed experiment on dune soil nitrogen economy.

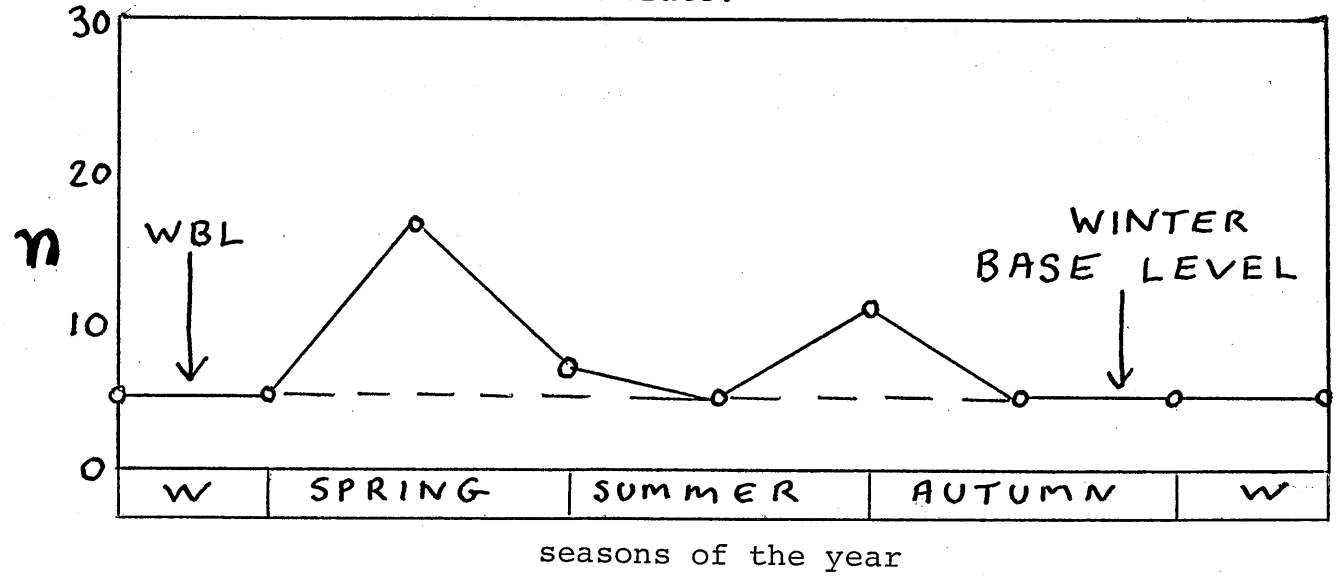
The seasonal variations in soil nitrate observed for poor chalky soils in the Chilterns (DAVY & TAYLOR 1974) were examined in detail in Chapter 1, section 10. The levels of nitrate measured were of the same order of magnitude as those obtained in the study at Hunstanton. However the seasonal variations observed by Davy and Taylor were in marked contrast (Graph 4.4A). They found maxima in May and November. The explanations advanced by the authors who were concerned with nitrifying bacteria are not applicable to the Hunstanton study. They considered that the May peak was due to rising temperatures leading to the breakdown of organic tissue from soil fauna killed by winter frosts. The second

Figure 4.1 (A) Model of seasonal variation in nitrate economy of a dune soil with Hippophaë scrub.



Key: N = nitrate contribution to the soil - arbitrary units.

(B) Model of seasonal variation in level of dune soil nitrate.



Key: n = nitrate level in soil arbitrary units

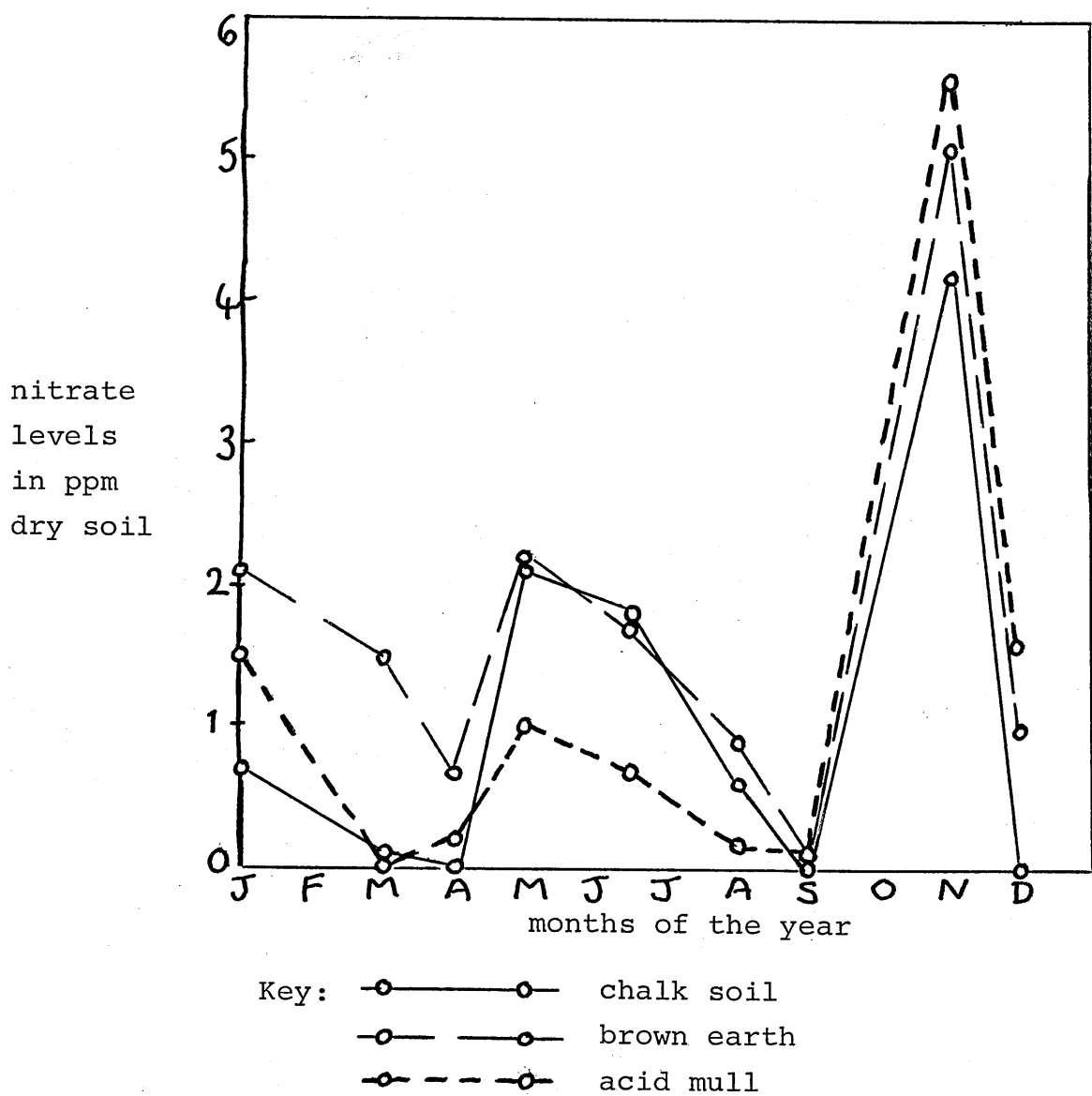
200

peak in November was due possibly to the breakdown of litter accumulating during the autumn just before the major temperature drop. Perhaps their most relevant comment is that soil water content, and dryness may have influenced the peaks and troughs of nitrification observed. The same might well be true of the degradation and release of nitrate from nodules of *Hippophaë* in the Hunstanton study.

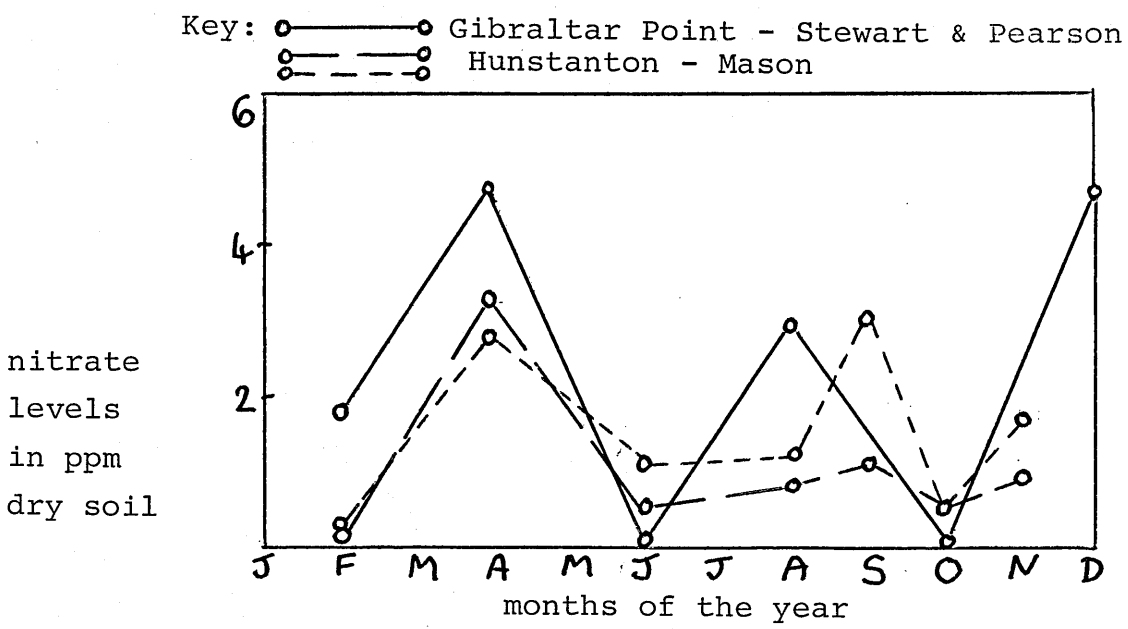
At Gibraltar Point, Stewart and Pearson obtained a picture of seasonal variation which, in broad terms, revealed a winter maximum and a summer minimum in nitrate levels within dune soil. At their site II, (scrub aged about 11 years) they obtained results very similar to those obtained in my study at Hunstanton, for scrub of about the same age, that is a peak in April and another in August-September. Their winter peak in December was not seen in the Hunstanton study as no samples were taken for this period, although the November results indicate a slight rise. (Graph 4.4B).

The supposed correlation with rainfall was also examined by Stewart and Pearson (discussed fully in Chapter 1. Section 6) but they found that although the summer rainfall maximum correlated with the August nitrate peak, the winter minimum for rainfall correlated with a peak for soil nitrate levels. (STEWART & PEARSON 1967). This suggests that there is probably some multifactorial causality at work, as suggested by the model in Figure 4.1, involving rainfall, temperature, decay, and plant growth, at least.

Graph 4.4 (A) Seasonal variation in nitrate levels in chalk soils (data from Davy and Taylor 1974)



Graph 4.4 (B) Comparison of seasonal variation in nitrate at Gibraltar Point, and Hunstanton. (Data from Stewart and Pearson 1967)



#### 4.1.2 Variation in nitrates with age of scrub

Examination of Graphs 4.2 and 4.3 shows that nitrate levels under older stands of *Hippophaë* are markedly higher at all seasons. The relationship between age of scrub and nitrate level is brought out more clearly in Graphs 4.5 and 4.6 where the increase is seen to be more or less linear, notwithstanding the problems of estimating scrub age and also the deviations from the means. The data from April and September were used because the nitrate values were highest, but the same pattern holds for most other months.

It is to be expected that the soil under the older scrub will contain more necrotic root nodules. If the presence of nitrate is due to the breakdown of these nodules, principally, then it too should be at a higher level.

#### 4.1.3 Differences between control sites and experimental sites

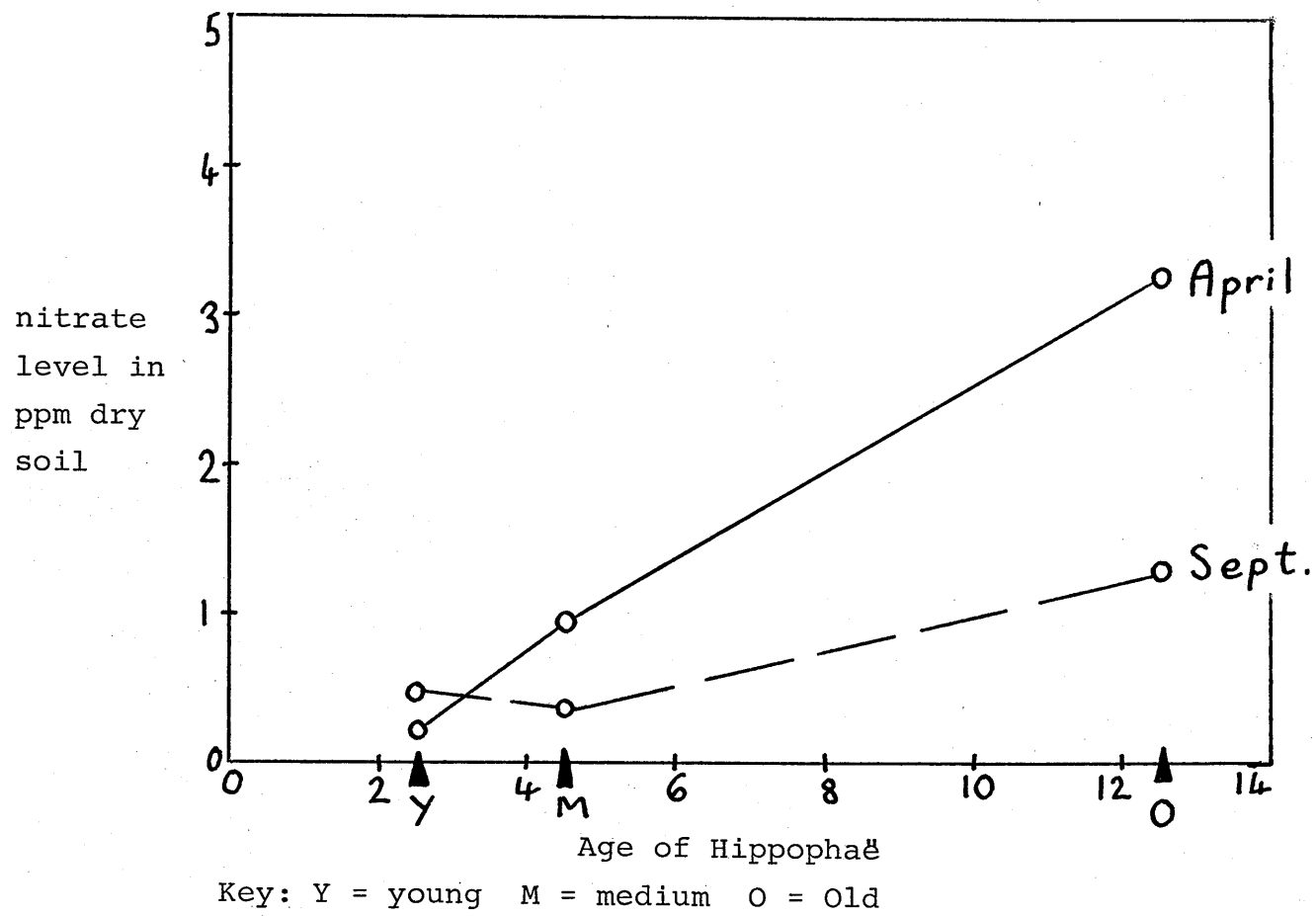
There is clearly some difference between the control and experimental sites as shown in Graphs 4.7 and 4.8. The data for the old scrub sites during the year were selected as the nitrate values were highest. In graph 4.7 the means for the controls initially exceeded the experimentals, and then the position reversed for the latter part of the year. It is not possible to tell whether this is a significant result or not, just by inspecting the graphs.

It is even less clear from Graph 4.8 as to whether the control/experimental difference is significant or not. The agreement appears good for April but very disparate in September. It

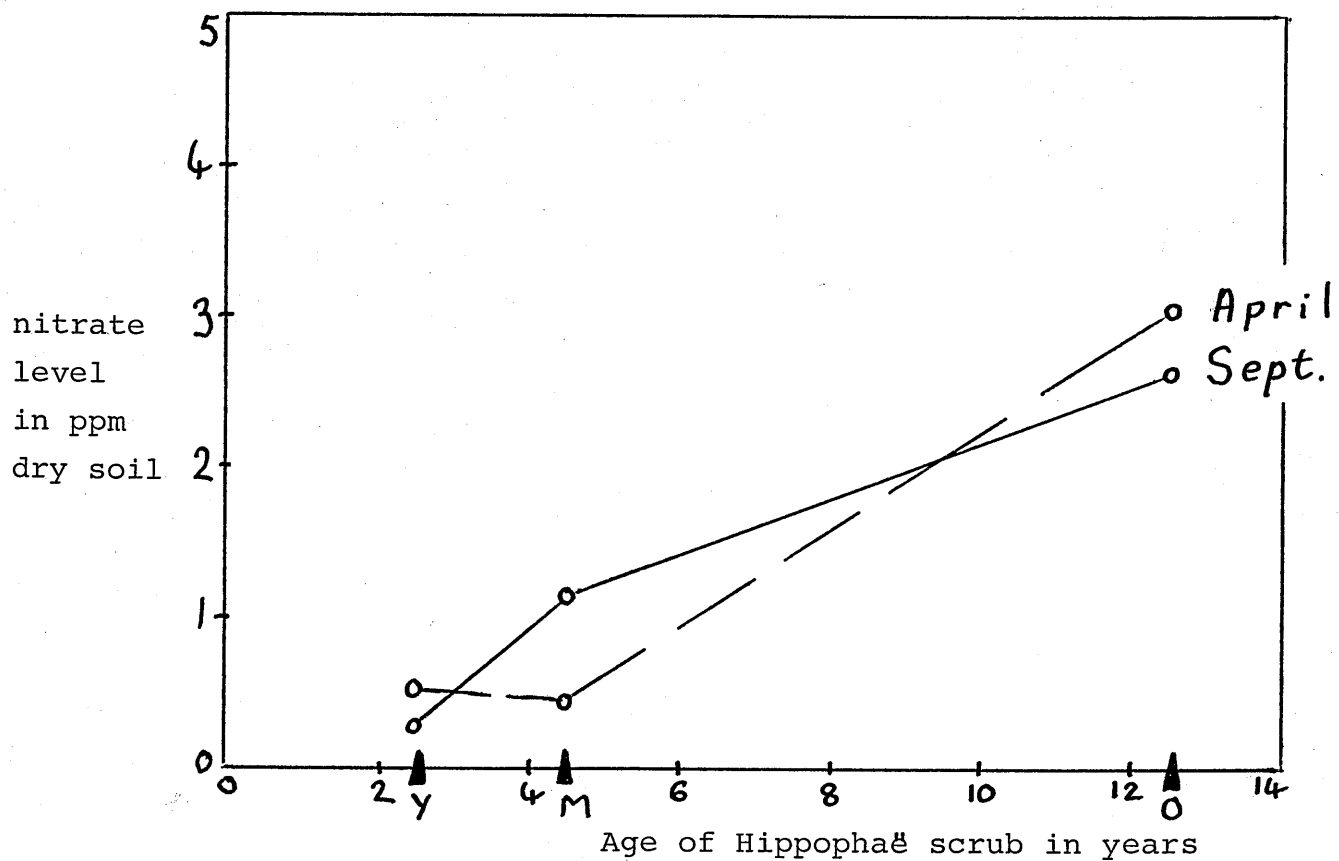


Variation in levels of dune soil nitrate with age of Hippophaë scrub. Graphs 4.5 and 4.6

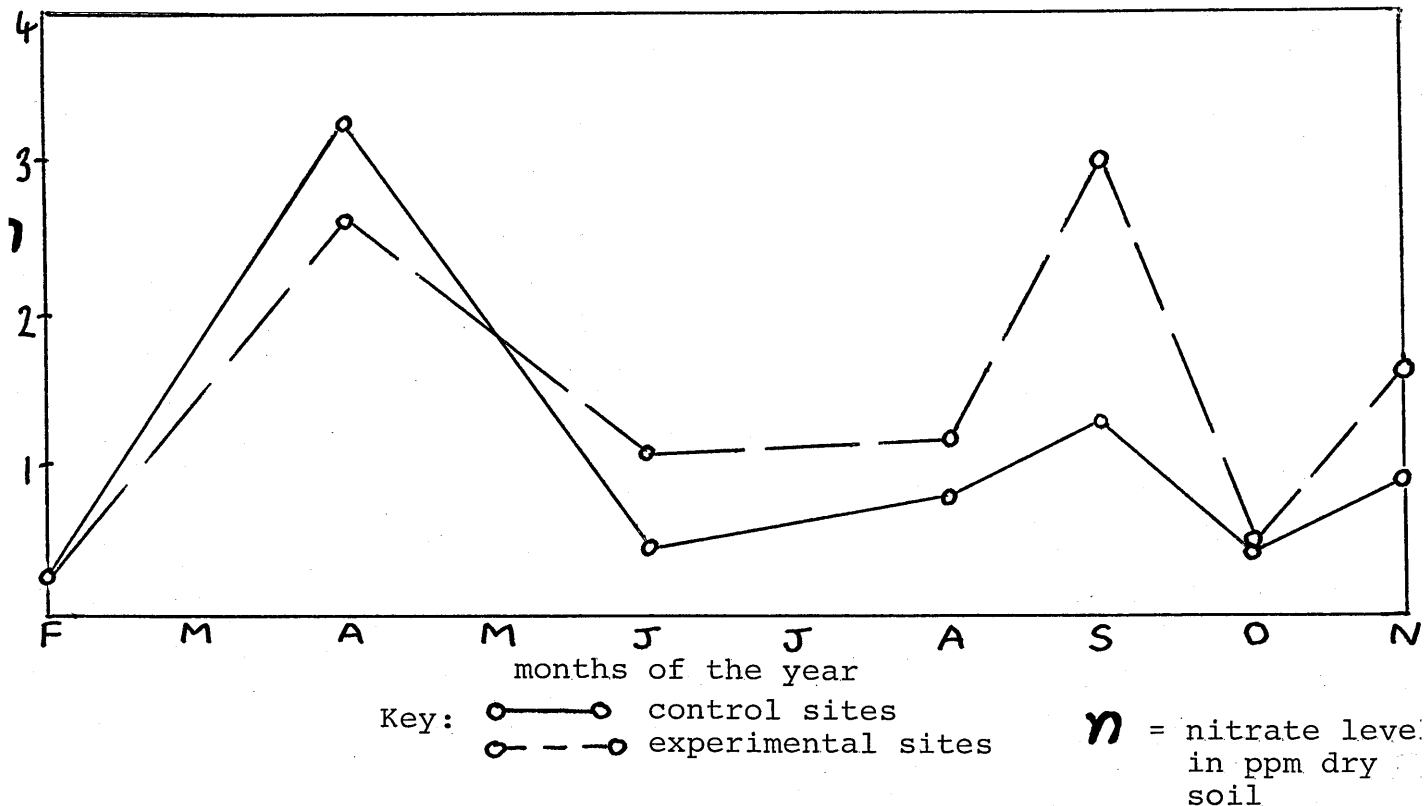
Graph 4.5 Control sites



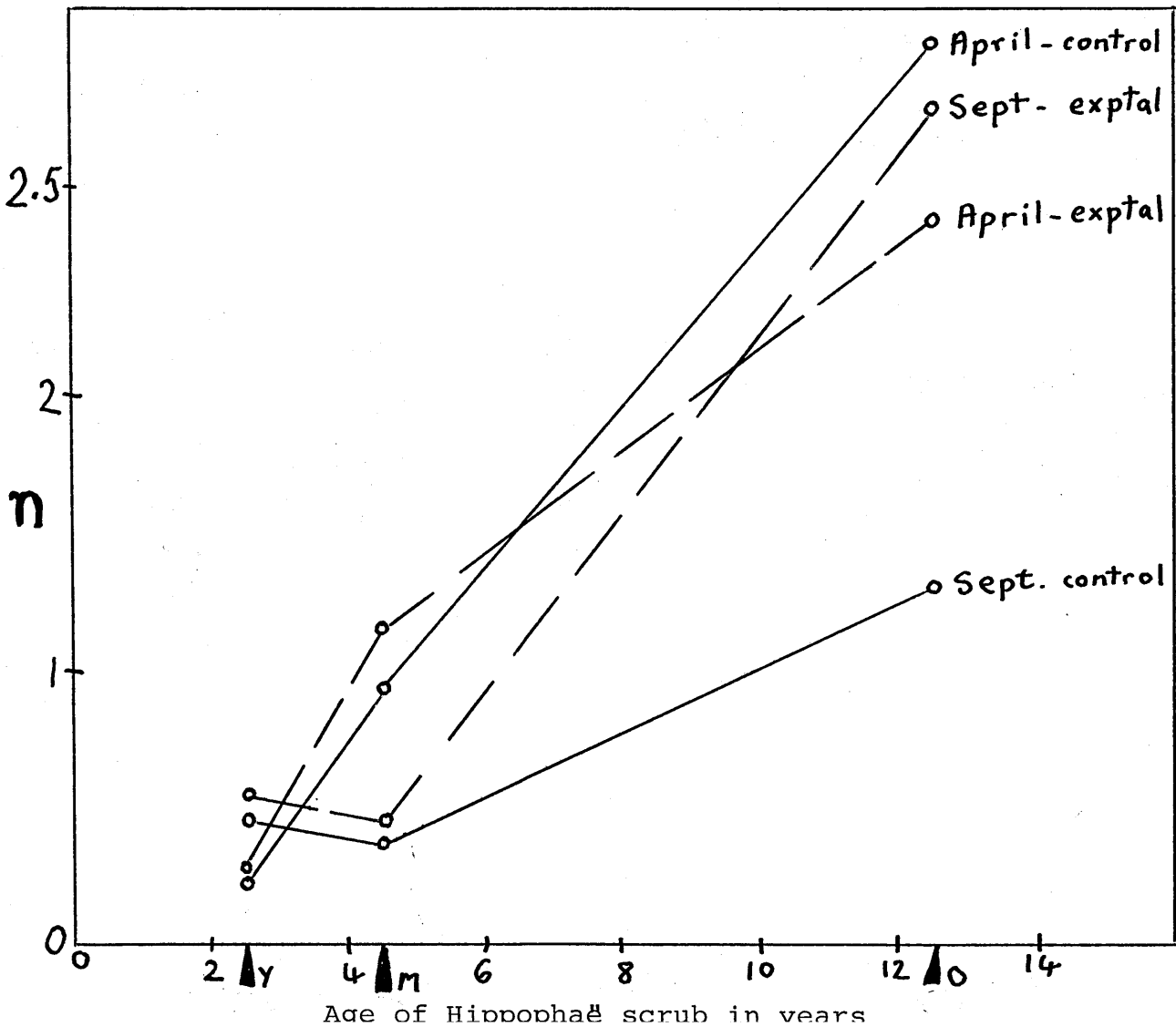
Graph 4.6 Experimental sites



Graph 4.7 Comparison of seasonal variation in dune soil nitrate levels at control and experimental sites within old Hippophaë scrub.



Graph 4.8 Variation of dune soil nitrate levels with age of Hippophaë scrub. Comparison of control and experimental sites



is necessary to use one of the standard methods of statistical analysis to assess the significance of any differences.

## 4.2 Principal project - Statistical analysis

The raw data have been subjected to just two methods of statistical analysis. When making use of arithmetic means of replicates it is usual to give the confidence limits as the standard error at a 5% level of significance, and this is the procedure here. In graphical presentations in section 4.3, the mean is indicated by a small circle and the confidence limits as bars above and below the intercept through the mean.

The formulation employed is:

$x$  = measured values of samples

$\bar{x}$  = mean

$n$  = number of samples

$$\text{Variance, } \sigma^2 = \frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}$$

Standard deviation =  $\sigma$

Standard error,  $\sigma_n = \frac{\sigma}{\sqrt{n}}$

Confidence limits =  $\bar{x} \pm \sigma t$

Values of  $t$  were selected at the 5% level of significance from Tables. (BISHOP 1966, CAMPBELL 1967)

The other statistical procedure was used to assess the significance of the difference between the means of various groups. As the number of samples was small (thirty five at each season, five for each site) the  $t$  test of W.S. Gossett ('Student') was selected for this purpose, in preference to the three-way analysis of variance where problems had arisen in obtaining a suitable residual from which to calculate the variance ratio,  $F$ . Most of the individual statistical components had already been calculated in determining the

standard error of the means.

The formulation employed in comparing two populations of data x and y are as follows.

$\bar{x}$  = mean of values of x

$\bar{y}$  = mean of values of y

n = total number of values on x

m = total number of values of y

$\sigma_x^2$  = variance of x     $\sigma_y^2$  = variance of y

The variance ratio of F is calculated

$$F = \frac{\sigma_x^2}{\sigma_y^2} \quad \text{or} \quad \frac{\sigma_y^2}{\sigma_x^2}$$

entering published tables of the variance ratio where  $v_1 = n-1$  and  $v_2 = m-1$  (if  $F = \frac{\sigma_x^2}{\sigma_y^2}$ ) the expected value of F at the 5% significance level was noted. When the observed value of F was calculated from:

$$t = \frac{\bar{x} - \bar{y}}{B} \quad \text{or} \quad \frac{\bar{y} - \bar{x}}{B} \quad \text{where } B = \sqrt{\frac{\sigma_x^2}{n} + \frac{\sigma_y^2}{m}}$$

When the observed value of F was less than the expected value t was calculated by a longer method.

$$\Sigma d_X^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n}$$

$$\sigma_T^2 = \frac{\Sigma d_X^2 + \Sigma d_Y^2}{n+m+2}$$

$$B = \sqrt{\sigma_T^2 \left( \frac{1}{m} + \frac{1}{n} \right)} \quad \text{and } t = \frac{\bar{x} - \bar{y}}{B}$$

#### 4.2.1 Comparison of experimental and control sites

This was the principal check on the whole study, because if the control sites were all significantly different from the experimental ones it would indicate that factors other than the sea-buckthorn influenced dune-soil nitrogen levels.

The t test was run on four sets of seasonal data to see if there was a significant difference between control and experimental sites. April and September gave the largest values for soil nitrate (see Graphs 4.2 and 4.3).

Though June and November showed low mean values they were chosen to provide a contrast. The statistics in the t test are summarised in Table 4.3.

Only two of the results indicate a significant difference between the means. These were for (i) medium-age scrub in June and (ii) old-age scrub in September. It is unlikely that these two results reveal a trend. In case (i) if there was a trend towards experimental/control differences it would also be observable in the old-age scrub in June, but this was not seen, even though the values are higher.

The contrast between experimental and controls in (ii) the old-age scrub in September shows up clearly even in graphs (see Graph 4.3) and it is not surprising that the t-test reveals a significant difference. Going back to the raw data in Table 4.1 it seems clear that there is one single high result, double the value of the other four. Such a high value could be a purely local effect. The only way to resolve such a problem is to have a larger sample population so that

local effects are much less important.

In general it seems clear that the null hypothesis is upheld, and there is no significant difference between control and experimental sites. This means that the leaf litter, and the droppings of animals appear to make a negligible contribution to the levels of dune-soil nitrogen in comparison with the effect of the sea-buckthorn bushes.

Table 4.3 Comparison of experimental & controls 'Student's' t test of significance

#### Hunstanton Data

KEY: F = Variance ratio

$t_o$  = observed value of t from data

$t_e$  = expected value of t from tables at 5% significance level

S.D. = significant difference between experimental and control sites

Season	Age of Scrub	F	$t_o$	$t_e$	S.D.
April	Young	9.00	1.52	2.31	No
	Medium	1.93	0.57	2.31	No
	Old	1.37	0.67	2.31	No
June	Young	7.90	1.41	2.31	No
	Medium	3.81	2.67	2.31	Yes
	Old	7.25	1.57	2.31	No
September	Young	1.11	0.71	2.36	No
	Medium	26.00	0.59	2.31	No
	Old	15.00	2.48	2.31	Yes
November	Young	2.35	0.33	2.36	No
	Medium	1.32	0.83	2.31	No
	Old	6.07	0.99	2.31	No

#### 4.2.2 Comparison of different ages of scrubs

Although Graphs 4.5 and 4.6 seem to reveal a clear relationship between the age of the sea-buckthorn scrub, and the levels of soil nitrate, given the limits of the standard error of the means it is worth checking to see if the t test indicates that the differences are significant. The data chosen are those for the maxima in April and September. Comparisons are made (a) between young and medium scrub (b) medium and old, and (c) young and old scrub.

The calculated statistics are shown in Table 4.4. In the comparison between young and medium age scrub (a) only one result out of four was significant, which is perhaps not surprising when recalling the small differences in Graphs 4.5 and 4.6. When comparing medium and old-age scrub (b) there were two significant results out of four, which is not as good as might have been expected. Even the comparison between young and old scrub (c) only gives three out of four significant results, where one might have expected that all would be significant. Obviously the extent of variation in a small number of samples does preclude absolutely clear-cut conclusions.

However the trend is quite clear, that with increasing age of sea-buckthorn scrub the level of dune-soil nitrate increases.



Table 4.4 Comparison of different ages of scrub 'Student's'  
t test of significance

KEY: as for Table 4.3

Season	Comparison	Treatment	F	t <sub>o</sub>	t <sub>e</sub>	S.D.
April	Young/Med.	Control	>1000	1.88	2.31	No
		Exptal	114	3.17	2.31	Yes
	Medium/Old	Control	10.8	2.43	2.31	Yes
		Exptal	15.2	1.84	2.31	No
Sept	Young/Old	Control	5390	3.45	2.31	Yes
		Exptal	437	3.11	2.31	Yes
	Young/Med.	Control	9.0	0.45	2.31	No
		Exptal	13.0	0.66	2.37	No
	Med/Old	Control	2.7	5.51	2.31	Yes
		Exptal	602	1.69	2.31	No
	Young/Old	Control	6.59	4.19	2.31	Yes
		Exptal	92	1.62	2.37	No

The preceding results indicate that there is no significant difference between the control and experimental sites as was expected. The problem with the other data is the small number of replicates. Given the lack of significance between controls and experimental sites it is thus possible to combine these and re-examine the age and seasonal differences.

Table 4.5 presents the contrast in age differences for four months, February, April, September, and November. During the first assay, the values for nitrate were very low and there is no significant difference between any of the age groups. In the later months however the differences are significant even at the 1% level.

Table 4.5      Comparison of different ages of sea-buckthorn scrub (combined experimentals and controls)

'Student's' t test of significance

Season	Comparison	F	t <sub>o</sub>	t <sub>e</sub> *	S.D.
February	Young/Medium	29	1.74	2.58	No
	Medium/Old	21	1.11	2.11	No
	Young/Old	2.76	1.79	2.11	No
April	Young/Medium	240	3.54	2.88	Yes
	Medium/Old	12.5	3.17	2.88	Yes
	Young/Old	1498	4.84	2.88	Yes
September	Young/Medium	4.98	0.78	2.90	No
	Medium/Old	40.4	3.89	2.88	Yes
	Young/Old	50.3	3.74	2.88	Yes
November	Young/Medium	29	1.05	2.90	No
	Medium/Old	21	8.15	2.88	Yes
	Young/Old	2.76	3.19	2.90	Yes

\* t<sub>e</sub>      expected value of t from tables at the 1% significance level

4.2.3      Comparison of seasons

The seasonal variation shown in Graphs 4.2 and 4.3 indicates a marked increase in April and to a lesser extent in September in soil nitrate levels. Was this a really significant result, bearing in mind the size of the standard error of the means? The t test will enable a check to be made on the significance of differences between successive sampling seasons, viz. (i) February and April, and (ii) August and September. As the oldest scrub had the highest values for soil nitrate these were used in the test.

Table 4.6 shows the statistics obtained, and both of the peaks of nitrate production are shown as significantly higher than that of the previous season in the old-age scrub.

Table 4.6      Comparison of successive sampling seasons  
'Student's' t test of significance

KEY: as for Table 4.3

Comparison	Treatment	F	t <sub>o</sub>	t <sub>e</sub>	S.D.
Feb/April	Control	600	3.43	2.31	Yes
	Exptal	340	3.13	2.31	Yes
Aug/Sept	Control	5.80	2.93	2.31	Yes
	Exptal	17	2.57	2.31	Yes

### 4.3 Nitrate levels in Dune Soils - Subsidiary Locations

Although far fewer analyses were carried out at the subsidiary locations these results were important as they placed the Hunstanton investigation within a wider context. They help to validate the techniques of analysis when it is evident that the sea-buckthorn scrub yields different results from other areas.

#### 4.3.1 Sea Beach Sites

The levels of nitrate were extremely low in this bare uncolonized sand as would be expected. Samples were taken at high tide, beyond the normal reach of the sea, and above the strand line of rubbish some of which is degradable material containing nitrogen.

Table 4.7 shows the four subsidiary locations, two with sea-buckthorn present, further inland, and two with none present. This data is contrasted with sea-beach analyses from Hunstanton displayed in Table 4.8. These were carried out on five occasions when collecting beach sand samples had been included as a regular part of the procedure.

The data were analysed to give a mean and standard error for each group of replicates (see Tables 4.9 & 4.10). This information was displayed in graphical form. It is clear from Graph 4.9A that there is a marked difference between the beach sand in the sites lacking sea-buckthorn (Braunton, Daymer, and Studland) and the higher values at Hunstanton and Gibraltar Point.

Table 4.7 Nitrate levels in dune soil at subsidiary locations - ppm dry soil

A. Hippophaë present

Location	Sea Beach	Young Hippoph.	Medium Hippoph.	Old Hippoph.
Braunton Burrows, Devon	.00	.01	.06	
	.00	.00		
	.00	.01		
	.00	.00		
	.00	.00		
Gibraltar Point, Lincs.	.50		.27	1.74
	.46		.58	1.56
	.45		.30	2.81
	.62		.28	0.62
	.50		.18	0.88

B. Hippophaë absent

Location	Sea Beach	Young Marram Dune	Mature Marram Dune	Dune Slack	Heather Scrub	Hawthorn Scrub
Daymer Bay CORNWALL	.04	.05		.04		.05
	.04	.05		.03		.07
	.02	.04		.05		.06
	.02	.09		.04		.07
	.02	.04		.04		.07
Studland Heath DORSET	.05	.05	.10		.06	
	.05	.04	.11		.05	
	.08	.04	.07		.14	
	.04	.04	.08		.06	
	.03	.04	.15		.04	

Table 4.8 Nitrate levels in sea beach sand -  
Hunstanton-Holme

Raw data as nitrate in ppm.

Sample Ref.	Season	Replicates				
100-104	June	.22	.04	.09	.04	.12
155-159	August	.10	.28	.16	.17	.59
191-195	September	.16	.13	.01	.31	.39
226-230	October	.04	.06	.07	.12	.60
261-265	November	.20	.09	.17	.14	.25

Table 4.9 Nitrate levels in sea beach sand -  
Hunstanton-Holme

Statistical analysis - mean and standard error

Season	Maximum	Mean	Minimum	$\sigma_n t$
June	0.194	0.102	0.010	0.092
August	0.503	0.260	0.017	0.243
Sept	0.387	0.200	0.013	0.187
Oct	0.474	0.178	0.000	0.296
Nov	0.245	0.170	0.095	0.075

Table 4.10 Statistical Analysis of nitrate levels in dune soil at subsidiary locations

The raw data were analysed to give mean and standard error. The results show the concentration of nitrate ion in ppm dry soil.

A Hippophaë present

Location	Statistic	Sea Beach	Young Hippo.	Medium Hippo.	Old Hippo.
Braunton Burrows, Devon	maximum mean minimum	- .00 -	.01*	.06 <sup>†</sup>	
Gibraltar Point, Lincs.	maximum mean minimum	.59 .51 .42		.51 .32 .13	2.59 1.52 0.457

B Hippophaë absent

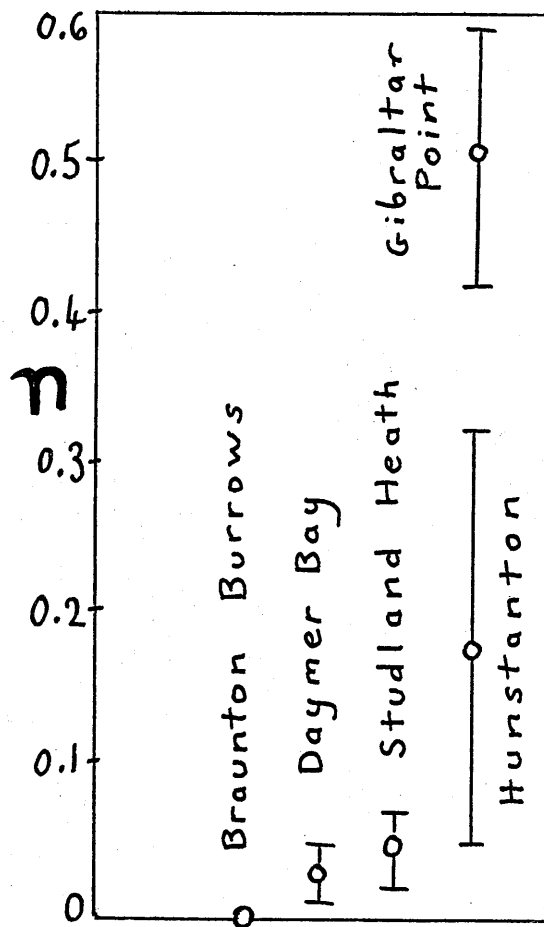
Location	Stat	Sea Beach	Young Marram Dune	Mature Marram Dune	Dune Slack	Heather Scrub	Haw-thorn Scrub
Daymer Bay, CORNWALL	max mean min	.04 .03 .01	.13 .05 .00	-	.05 .04 .03	-	0.08 0.06 0.03
Studland Heath, DORSET	max mean min	.07 .05 .03	.05 .04 .03	.14 .10 .07	-	.12 .07 .02	-

\* 2 samples only

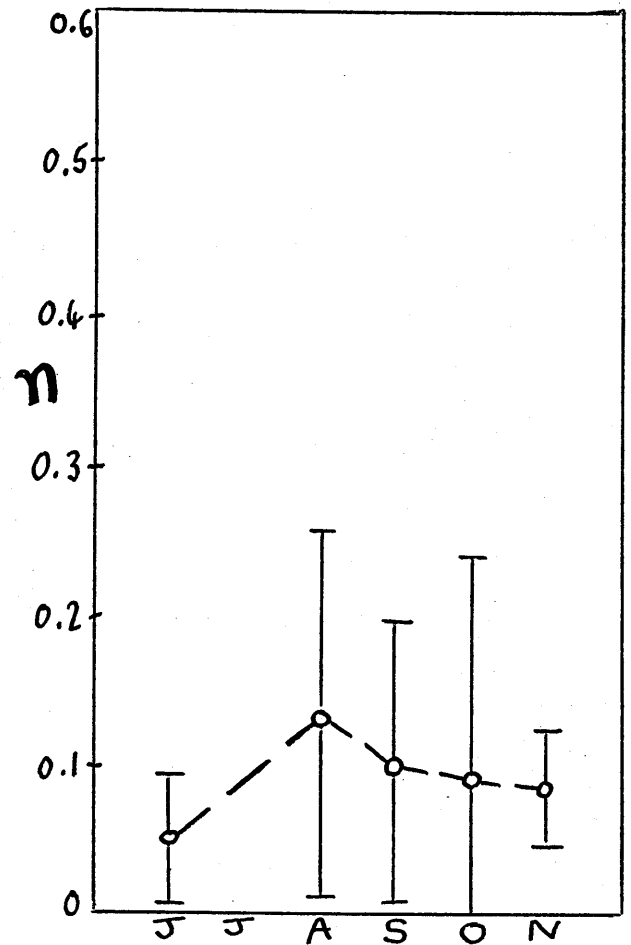
† 1 sample only

Graph 4.9 Levels of dune soil nitrate at principal and subsidiary locations

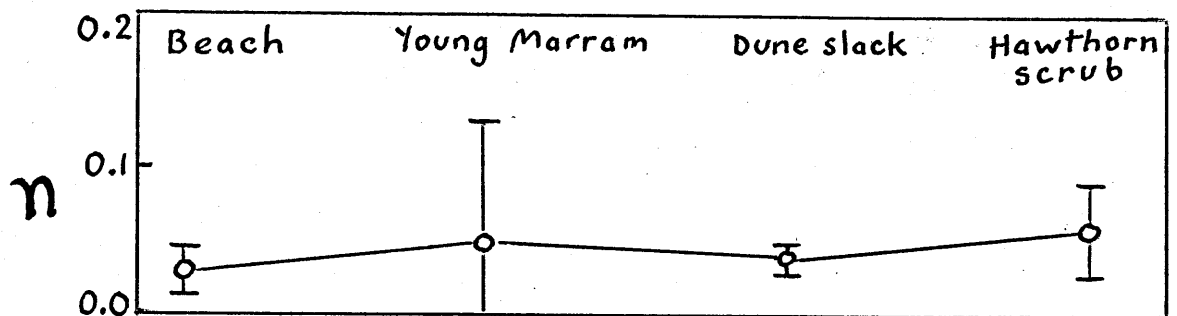
Key:  $n$  = nitrate level in ppm dry soil mean and standard error



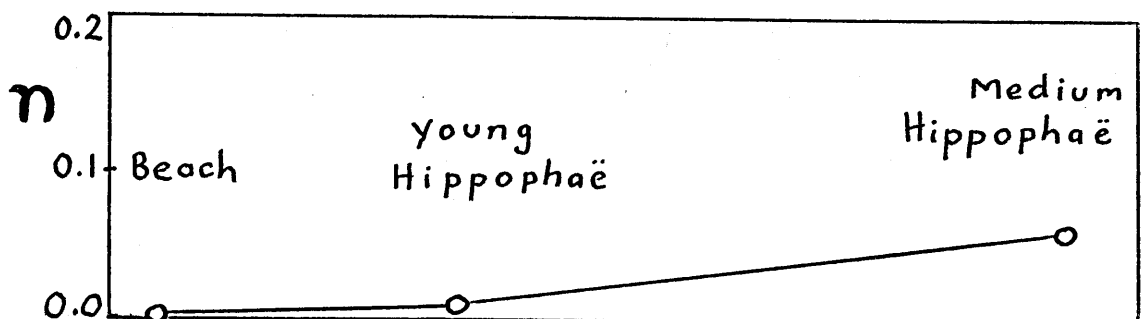
A. All sea beach sites



B. Hunstanton beach seasonal variation



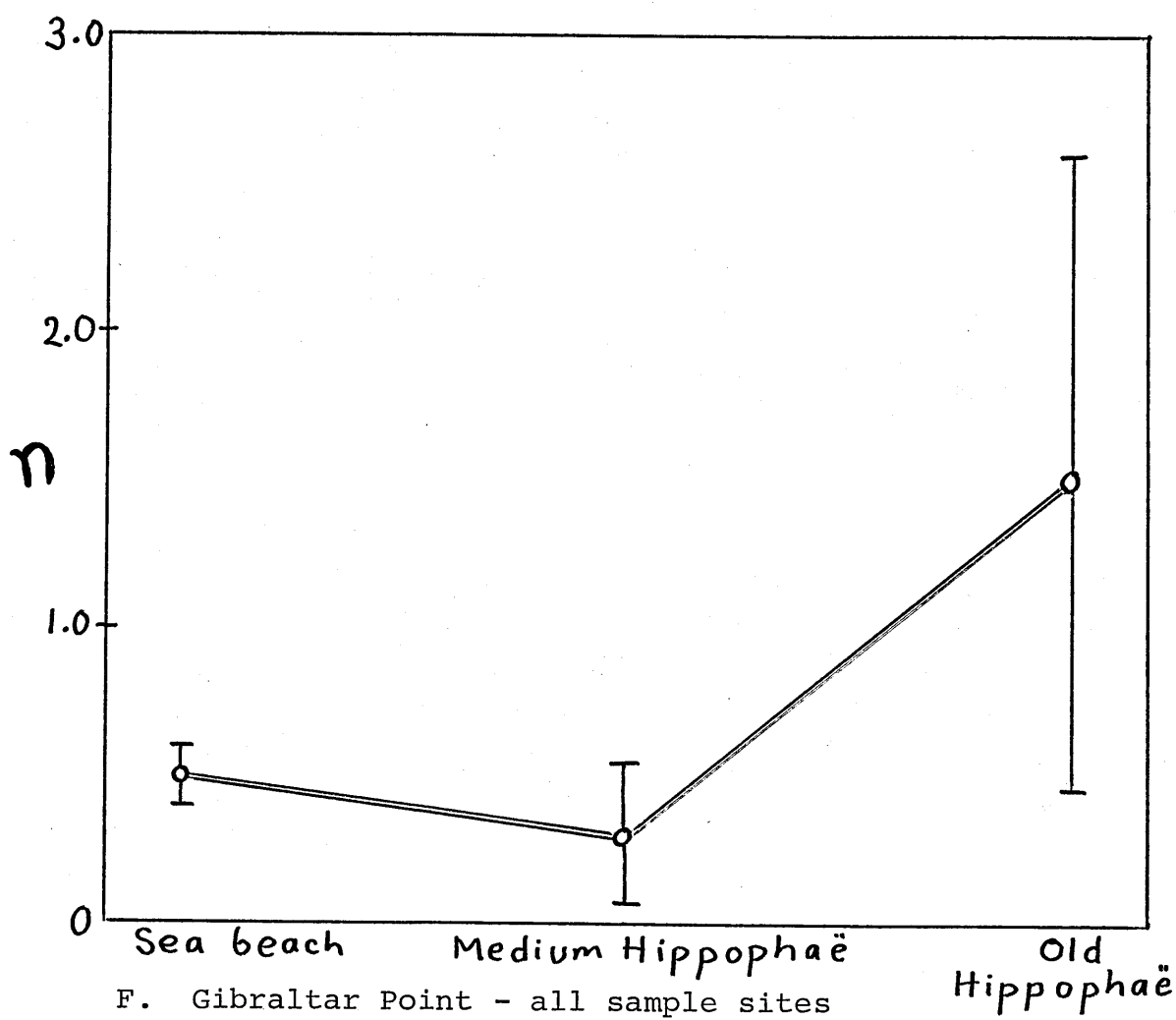
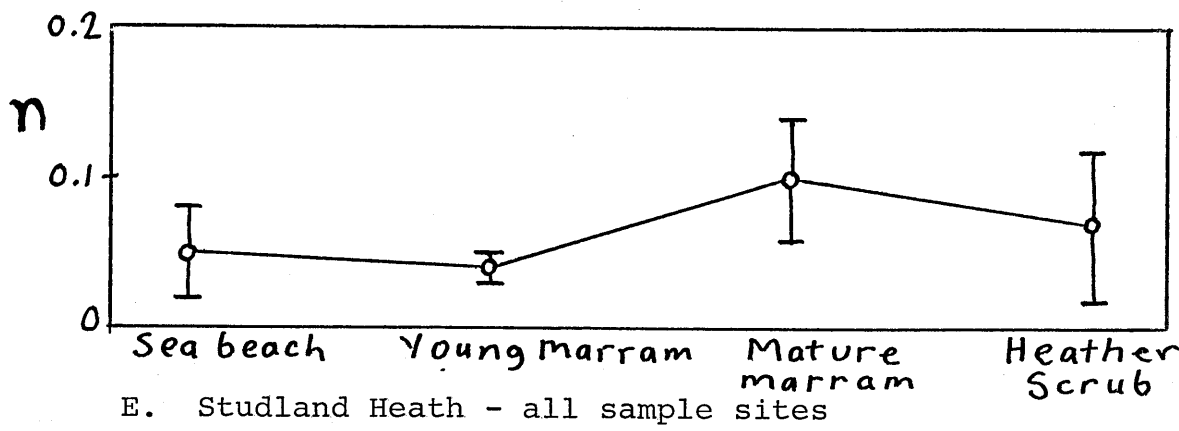
C. Daymer Bay - all sample sites



D. Braunton Burrows - all sample sites



Graph 4.9 (continued)



n = nitrate level in ppm dry soil, mean and standard error.

It is not easy to offer a clear explanation for this. In all cases the dune soil was bare of obvious plant life, no marram and none of the other early colonizers was present. Neither was there any appreciable quantity of strand-line litter as the sampling was above this level.

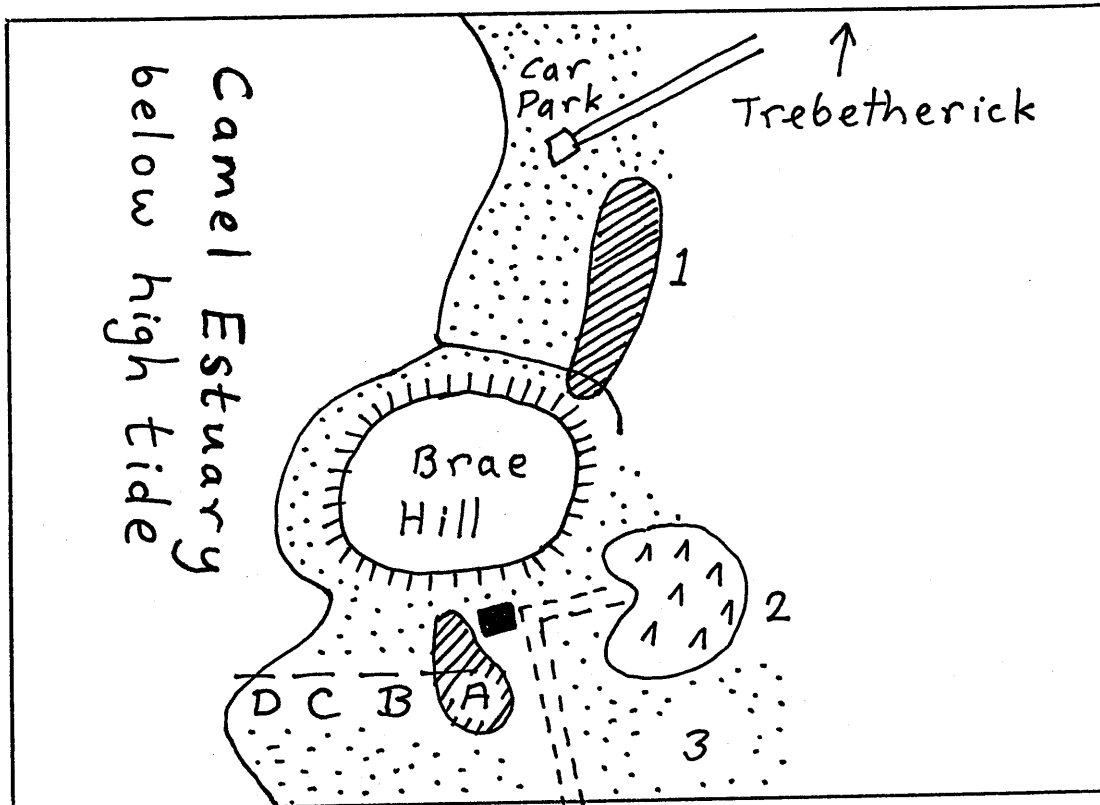
The samples were taken at locations several metres apart to avoid the possibility of local contamination affecting nitrogen levels. The quantities of nitrate measured were small, a few parts in ten million, but the data were consistent, as the standard error indicates. No nitrate was measurable at Braunton in any sample. No values at Daymer exceeded 0.04, and none at Studland exceeded 0.08. Yet at Gibraltar Point none of the values was less than 0.45. It is interesting to note how much higher the values are at Gibraltar Point compared with Hunstanton.

The most obvious hypothesis is that the presence of a large stand of sea-buckthorn influences nitrogen levels in the whole of the ground water which percolates through the dune system. At Gibraltar Point the sea-buckthorn is very extensive, whereas at Hunstanton-Holme it is more modest in area. This could account for the higher nitrate levels recorded at Gibraltar Point. It may seem surprising that the sea-buckthorn stands should create effects at a distance from where they grow, but dune systems are actually very damp habitats, despite the dryness of the surface sand. The water table is often no more than a metre below the surface, and the deeper dune slacks may contain small pools of water. In such a wet soil, with such a light structure, it seems quite reasonable to expect continual water percolation bringing about a wider distribution of soluble substances

from one part of the system to another. The nitrogen-fixing capability of sea-buckthorn creates more widespread effects on dune-soil fertility than has been appreciated so far.

At Hunstanton, the beach soils were analysed over a six-month period, and the means reveal a slight variation during this time. (Table 4.9). However, the confidence limits indicate that the variations are too small to have any real significance. (Graph 4.9B).

The idea that sea-buckthorn stands may influence the sand of the beach can remain no more than a hypothesis without a much fuller investigation. It is possible that there may be seasonal variations in the nitrate levels of beach sands although the data from Hunstanton do not support this. There may be seasonal or constant differences in the nitrate content of the sea-water off these beaches. It has been suggested that a relationship exists between the nitrate levels of sea-water and beach ground water. (PUGH 1976). The latter value is usually higher "indicating a dynamic equilibrium between input and utilization" (p.182). Although Pugh's experiments were made with a model beach some 0.4 m high and 2.6 m wide interacting with natural sea-water of the Menai Straits it seems clear that in a real beach the level of nutrients may well depend upon complex interactions between the sea, and habitats adjacent to the beach. It is certainly known that the water table of dunes rises and falls in response to tidal movements of the sea (CHAPMAN 1964 p.172) and also in response to rainfall, giving a winter maximum when rain is plentiful and plant transpiration is minimal. (RANWELL 1959). These mechanisms may be sufficient to explain the movement of



Daymer Bay, Cornwall

Figure 4.2

# KEY



loose sand



scrub

1. thick scrub, small trees up to 5 m
2. small conifer plantation
3. wide expanse of marram dunes, range of grasses, slacks not much different from dune tops except for presence of legumes. Scrub less dense than 1, and no *Prunus* present.

## A-D Soil Collection Sites

A Hawthorn Scrub  
*Crataegus monogyna*  
*Prunus Spinosa*  
*Poa annua*  
*Dactylis glomerata*

*Clematis vitalba*  
*Agrostis tenuis*  
*Holcus lanatus*

B Dune Slacks  
*Anthyllis vulneraria*\*  
*Dactylis glomerata*  
*Poa annua*

*Lotus corniculatus*\*  
*Agrostis tenuis*

C Marram Dune  
*Ammophila arenaria*

D Sea Beach - no plants

\* nitrogen-fixing legumes

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nutrients from beneath the scrub, to the sea beach.

#### 4.3.2 West Coast - Daymer Bay, Cornwall

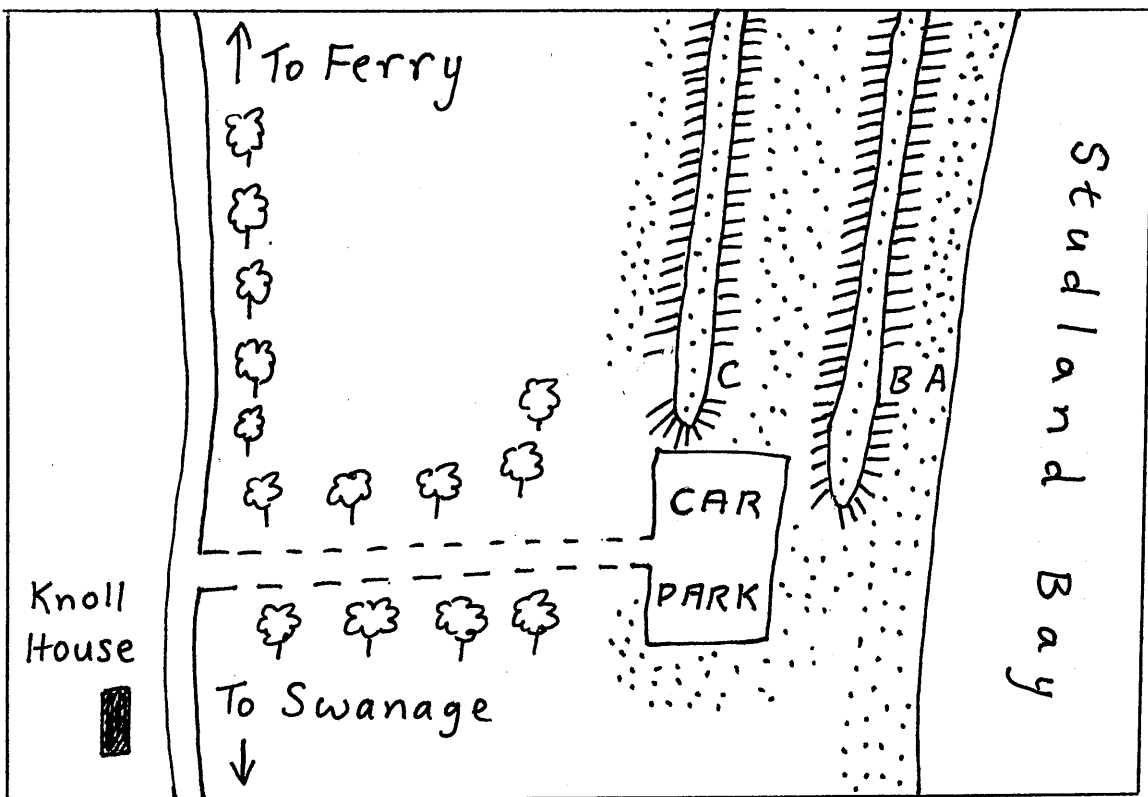
The interest of this site lay in the existence of a dune scrub which lacked sea-buckthorn, a small dune slack with leguminous colonizers, and a typical young marram dune. Samples were taken from these three regions and the sea beach. As Table 4.10 shows the mean values of nitrate were all very low. In Graph 4.9C there is clearly no significant difference between the four sites. The mean level of nitrate for the scrub is almost identical with the other three.

It seems evident that the presence of nitrogen-fixing legumes colonizing the dune slacks had little effect on soil nitrate. Similarly it is clear that nitrates do not accumulate in quantity under young dune scrub which lacks sea-buckthorn.

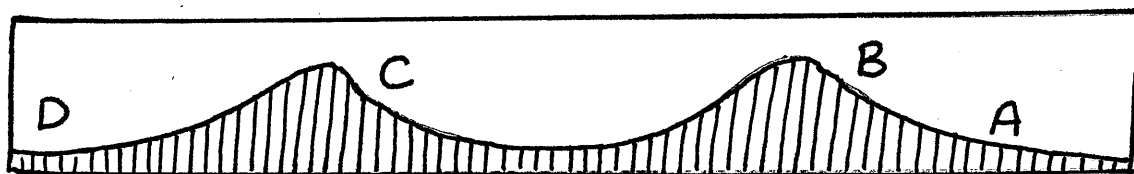
A map of the site and details of the flora present is included.

#### 4.3.3 West Coast - Braunton Burrows, Devon

This was a site from which sea-buckthorn had been cleared. The levels of soil nitrate as indicated in Table 4.7 were very low, even beneath the few bushes that were discovered. Graph 4.9D shows a slight rise from sea beach to bushes but with such a small number of samples it is not a significant result, though in line with measurements obtained at Hunstanton and Gibraltar Point.

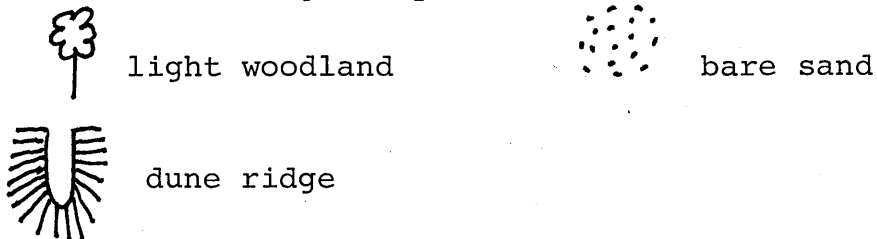


Studland Heath, Dorset - Figure 4.3



Section through sample sites

KEY



A Sea Beach

*Ammophila arenaria*  
*Honckenya peploides*

*Carex arenaria*

B/C Marram Dunes

*Ammophila arenaria*  
*Honckenya peploides*  
*Veronica serpyllum*  
*Hieracium* sp.  
*Epilobium* sp.

*Carex arenaria*  
*Elymus arenaria*  
*Calystegia soldanella*  
*Cladonia* sp.  
*Potentilla argentea*

D Heather Scrub

*Festuca rubra*  
*Erica tetralix*  
*Pteridium aquilinum*  
*Ulex europaeus*  
*Salix* sp.  
*Cladonia* sp.

*Festuca ovina*  
*Erica ciliaris*  
*Calluna vulgaris*  
*Betula pendula*  
*Parmelia* sp.

#### 4.3.4 South Coast - Studland Heath, Dorset

This is an extensive heathland of stabilized dunes which have accumulated over the last three centuries as outlined in Chapter 2, section 4. Sampling was limited to four distinct sites as indicated on the accompanying map, with floristic details.

The nitrate levels are all low, even on the thickly vegetated heather scrub, so the breakdown of litter at this stage does not seem to contribute much to the soil. The highest level is that of the mature marram dunes and the result does appear (Graph 4.9E) to be significantly different from the more poorly colonized areas. This might be due to the effects of nitrogen-fixing algae from the leaf-sheaths (ABDEL WAHAB & WAREING 1980) but this mean value is not up to the mean values obtained at Hunstanton or Gibraltar Point. The twenty samples confirm the general finding that nitrate levels are very low in dune soils, even when there is quite a thick cover of vegetation.

#### 4.3.5 East Coast - Gibraltar Point, Lincolnshire

It was expected that a survey of soil nitrate at Gibraltar Point would show great similarities with the results emerging from the study at Hunstanton-Holme because of the extensive sea-buckthorn scrub which is present there. Only fifteen soil samples were collected from Lincolnshire for analysis but this was sufficient to demonstrate the similarity. The data are given in Table 4.7.

It is evident that the difference between the sea-beach and the medium scrub is not significant, given the size of the

standard error (Graph 4.9F) but the difference between the two ages of scrub is significant. It is instructive to compare the results from Gibraltar Point with similar results from Hunstanton. The samples from Gibraltar Point were collected on 14.7.81 but as no samples were collected in July in Hunstanton comparison had to be made with the June and August collections.

The results concerning Hunstanton are displayed in Table 4.11 and are shown in Graph 4.10. What seems clear is that the two locations yield broadly similar results, and a gradual increase in nitrate from the beach to the sea-buckthorn stands is evident. A much larger series of samples would be needed to try to establish whether extensive stands of old buckthorn create significantly higher levels of nitrate compared with much smaller stands such as those occurring at Hunstanton-Holme.

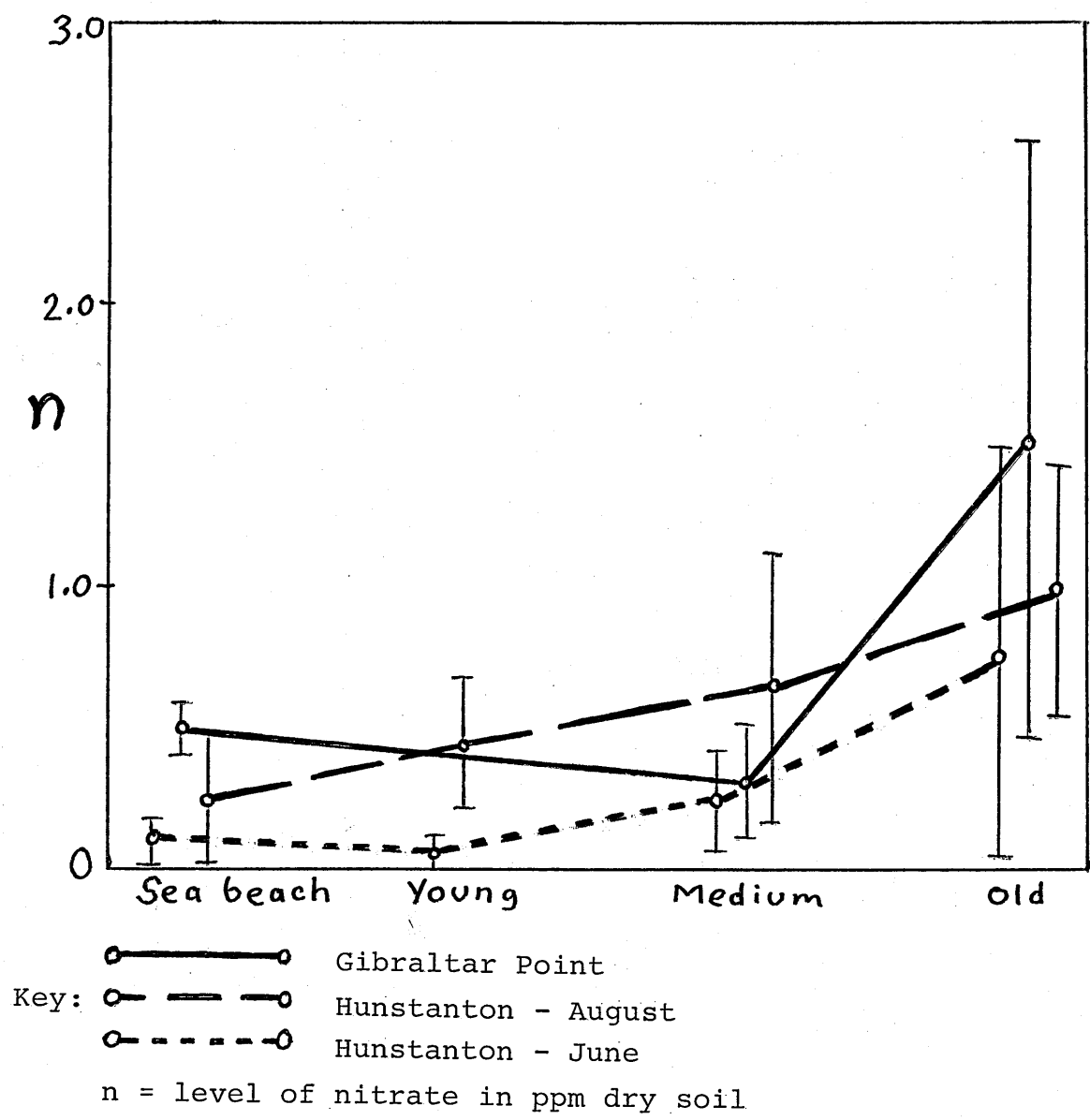
Table 4.11 Mean levels of nitrate in dune soils -  
Hunstanton-Holme

Season	Age of Scrub	ont	maximum	mean	minimum
June	Young	0.084	0.14	.06	0.00
	Medium	0.176	0.43	.25	0.07
	Old	0.718	1.49	.77	0.05
August	Young	0.234	0.69	.46	0.23
	Medium	0.475	1.13	.65	0.17
	Old	0.437	1.43	.99	0.55

with 9 degrees of freedom the value of the t-statistic at a 5% significance level = 2.26



Graph 4.10 Comparison of dune soil nitrate levels measured at Gibraltar Point, and Hunstanton - in Hippophaë scrub of different ages.



#### 4.4 Summary of conclusions

This series of experiments show that *Hippophaë rhamnoides*, L. makes soluble nitrogen in the form of nitrate ion, available within the soil for any later colonists of the dunes. The effect is probably a result of the nitrogen-fixing capabilities of the root-nodules and their later disintegration.

It seems clear that *Hippophaë* roots are the cause of the nitrate levels, because other types of dune scrub, like hawthorn do not produce this effect. Neither does the subsurface nitrate seem to be due to downward percolation from above.

The older the buckthorn scrub, the higher the level of nitrate, probably because there are more root-nodules under old scrub, and the disintegration of the older nodules is more complete.

Some variation in nitrate levels was charted through the year. Why it should be highest in April rather than midsummer is uncertain. The effect does not seem to be due to temperature alone, and remains inexplicable. It would be necessary to carry out a fuller investigation to determine whether the seasonal variation is a universal effect, or just a local variation in the Holme area in 1980. A larger number of replicates would be necessary for statistical validity than were used in this study. It would also be necessary to continue the monitoring of nitrate over several years to see if the April peak is a regular occurrence. A further possibility is that the nitrogen produced by seabuckthorn nodules affects the ground water of the whole dune system so that nitrates can reach the sea beach by percolation.

Chapter 5: The wider significance of the  
Sea-Buckthorn

At the conclusion of this study I want to turn from the technical details of dune soil nitrogen to briefly examine some general issues in connection with sea-buckthorn.

### 5.1 Management

Opinion remains divided on the appropriate management policy for the sea-buckthorn when it occurs on estates or reserves. Whether it should be eradicated, tolerated or encouraged is still an open question. In some cases, formerly thick stands of *Hippophaë* have been completely destroyed, as in the Braunton Burrows Nature Reserve in North Devon. Despite a strenuous eradication programme using volunteer labour it was clear in 1980 that a few specimens were still in existence and were propagating. In an extensive reserve like this constant inspection and effort is needed to prevent *Hippophaë* from recolonizing the area.

On the other hand, at the Gibraltar Point Reserve on the Lincolnshire coast there remains one of the most extensive thickets of sea-buckthorn in the country. It has been seen as a useful species for the control of public access. As it forms a literally impenetrable barrier it has been possible to allow public access to the beach by means of a limited number of planked walkways, and this has the additional merit of protecting the open dunes from damage. The dense thickets provide a safe haven for birds, with nest sites, shelter, and berries for food in autumn and winter. Across the Wash in Norfolk the very much smaller stands of sea-buckthorn at the Holme Nature Reserve, and the Holme Bird Observatory are encouraged for just the same reasons.

This particular argument about sea-buckthorn is part of a much bigger debate about whether habitats should be "conserved" or preserved in a fixed state or whether they should be allowed to develop naturally. It is worth reflecting that stasis, except in the climax vegetation of a habitat, is not "natural." The usual situation is one of a continuing succession. We may expect a dune system to continue to grow by accretion from offshore sand-bars, and a complete successional series from bare dunes at the sea's edge to the climax woodland further inland may be anticipated.

However, the sea-buckthorn does tend to upset what is considered the "typical" dune succession because it is an early colonizer and once established soon creates an extensive scrub largely devoid of all the variety of plants expected on grassy dunes, dune swards and dune slacks. In order to preserve these regions of rare plants it is necessary to eradicate the sea-buckthorn. It must be said that although the sea-buckthorn is a native plant it has not colonised every dune system in the British Isles and it is particularly unsuccessful on the acid dunes with low calcium levels in the soil. Even when it has spread successfully on dunes it does not always take over completely, and swards and slacks can still be found eg in Holme, Hunstanton, or Gibraltar Point.

This problem, of vigorous woody shrubs taking over a habitat, is not confined to the sea-buckthorn alone. The rhododendron group of plants became very popular in Victorian times as more and more new species were discovered in South and Central Asia. They were widely dispersed around Britain

in gardens, parks, and country estates, where some species escaped to grow wild in an uncultivated state. They were tolerant of poor soils, cold conditions, and occasional frosts. Compared with the mountains of Central Asia where so many of them grew naturally, the British Isles represented a very mild challenge to their colonizing ability and fortitude.

One species *Rhododendron ponticum* L., first introduced in 1763 and now widely found as a "wild" plant has begun to colonize the sand dunes in Winterton, Norfolk. (FULLER and BOORMAN 1977). In this case, as *R. ponticum* is such a recent arrival to the British Isles, the ethical dilemma or whether to eradicate or not, is perhaps less difficult than that posed by a native species like the sea-buckthorn. The authors concluded that *R. ponticum* L., was useful in providing cover for birds, and barring public access, and consequently "served to protect wild life interests in certain situations", (p. 93 FULLER and BOORMAN 1977) but nevertheless they advocated some degree of control to prevent widespread invasion of the habitat.

This dilemma of toleration or eradication of invasive species hinges upon the prevailing attitudes to the importance of rare species. Professional biologists have always taken an interest in rare species for the light they shed on the workings of evolution in the appearance of new species or the extinction of old ones. The ecological history of a particular region may be clarified by a study of its populations of relict species.

Frequently rare species have a certain news value, or their discovery has provided an interesting story, as in the case of Ginkgo, Latimeria, Père David's deer, or the Przewalski horses. The current popular view seems to be that common species are uninteresting or even a nuisance while the rarities are what is really important in ecology. This idea, though not stated explicitly by popularizers, journalists, and the amateur conservation lobby, seems to be implicit in their thinking. It becomes almost impossible to moot any kind of new development without someone discovering that the area in question contains the sole surviving members of a very rare species.

It is worth reflecting why a particular species is rare. Taxonomic biologists are well aware that rarity is, in fact, very common. Rarity and its natural concomitant extinction is the normal end of over ninety per cent of all animal and plant species. This is a logical outcome of the theory of natural selection, and it is the common experience of palaeobotanists, and palaeozoologists. If this were not so there would be no room for our modern species today.

Species are rare because they are unsuccessful, in competition with related species. Either as newcomers they have not been able to displace established species or as old species they are heading for extinction. There is confusion in the way the word "rare" is used both in biology and in collecting. A stamp, book or coin becomes valuable and sought after when rare, but this concept from the world of commerce is almost the exact opposite of the evolutionary value of rarities in biology. It is the common species which

are of value in that they are so successful.

In the light of these considerations should we then destroy the sea-buckthorn or the rhododendron because they are so successful so that a few dozen rare species might survive on the dunes? If a species is really common it will, of course, do little harm to its overall success if it is severely controlled to allow the survival of other species, admittedly unsuccessful, but inherently interesting and worthy of study. Success, moreover is a relative concept. It is a dynamic condition. It comes and goes. Some of the success of *Rhododendron ponticum* L. is due to the absence of the normal parasites and grazers from its natural home in S.E. Asia when it was imported into Britain. In a sense its success is artificial in that it has been influenced by human intervention. Even the present success of *Hippophaë* is artificial in that it has been initiated by the deliberate introduction into Britain in 1954 of the myxomatosis virus from South America. This decimated the rabbit population of Britain and as a consequence shrubs began to develop on a range of hitherto open habitats from chalk grasslands to dune systems.

It was at this point that the full significance of rabbits in keeping down scrub development by grazing was realised. Once *Hippophaë* was well established its invasive potential emerged clearly. A Dutch writer has identified a parallel situation in her own country. In the Netherlands Delta region "until the beginning of the twentieth century the dunes were intensively grazed by cattle. When grazing was stopped the scrub developed rapidly" (SLOET VAN OLDRUITENBORGH 1976 p. 104).



Holland has a much more extensive buckthorn scrub than Britain but even here the effects of grazing by stock, and by rabbits have been found to be quite remarkable once controlled experiments were undertaken after the rabbit population had re-established itself in the 1960s. (BOORMAN and FULLER 1973).

The whole issue of dune habitat management was given clear guidelines in a report to the Nature Conservancy following a comprehensive survey of sea-buckthorn around the coasts of the British Isles. (RANWELL 1972a). The author proposed a detailed compromise solution which recognised both the value of the plant as well as its drawbacks. He had observed that "Hippophaë has the capacity to replace most of the remaining range of semi-natural dune communities in the British Isles " (p. 11). A strong argument for some measure of control.

However sea-buckthorn is by no means a common plant in this country and it only flourishes naturally within dune systems so that some attempts at preservation are necessary. "The rich dune scrub association is not well represented in Britain and the full seral range is apparently not present on any reserve. The reason for this is not understood but it is probably related to over-grazing by rabbits in the past and by an early use of the fixed dune as arable land." (RANWELL 1972a p. 6).

The programme of management for Hippophaë put forward by Ranwell included preservation as well as eradication and in one case introduction (Dee estuary). The table below indicates the types of action recommended by him, with data

compiled from the Appendix to his report (pp. 46-49)

Table 5.1 Management of sea-buckthorn (RANWELL 1972 a)

Action	Sites
Allow it to remain, but control it	10
Eradicate it	7
Prevent it becoming established	24
No action needed	2
Action dependent on future developments	2
Introduce it	1
TOTAL	46

The aesthetic and control possibilities of *Hippophaë* at inland sites are becoming more widely known. It is advertised in the catalogues of nurserymen who specialise in more unusual species. For example, in the new Borough of Milton Keynes in Buckinghamshire *Hippophaë* has been planted extensively on roundabouts and landscaped road margins. It is too early to see if its invasive potential will prove to be a disadvantage, but given its inability to cope with heavy shade it is unlikely to fare well in competition with the common wild plants of the area. More local authorities are likely to realise the advantages of sea-buckthorn as a cheap but beautiful fence with no maintenance problems.

## 5.2 A reference species

Our Western European names for *Hippophaë* - sanddorn, duindoorn, sea-buckthorn, reflect the fact that it is largely confined to coastal sands here, and it is easy to overlook the inland locations where it occurs, with poor soils low in nitrogen, and more importantly free of shade-producing competitors. The colonizing ability of *Hippophaë* on poor soils, followed by a gradual change to a woodland of more typical trees and shrubs has an important ecological significance. It is so characteristic that it can be used as a standard marker of the transition between bare soil and open woodland in the history of succession in a given region. This history of succession is contained within cores obtained from large accumulations of peat, or in the pollen grains trapped within sediments cumulatively deposited over long periods in calm lakes.

Consequently in the pollen analysis of sediments from lacustrine deposits a band showing a high level of *Hippophaë* marks the transition from the periglacial to the post-glacial period - from the time of ice-bound landscapes, with sterile soils of rock-flour to the early succession towards coniferous woodland.

Scandinavia, among the last regions in N.W. Europe to be freed from glaciation, provides ample material in the way of undisturbed lake sediments for research into its past ecological history. As an illustration, one site in N.E. Finland yielded sediments with pollen demonstrating a periglacial period dominated by dwarf birch (*Betula nana*) and a few species of herbs. The rapid spread of *Hippophaë*

occurred at  $11,420 \pm 300$  years B.P., giving way to a light birch woodland with a ground flora dominated by ferns.

(REYNAUD 1976).

The sum of many such records reveals a picture of *Hippophaë* "spreading northward as the ice-front retreated, but retreating itself before the spread of the forests" (RANWELL 1972 a p. 4). The present distribution of *Hippophaë* populations in Europe represents a series of "islands" in mountain and coastal regions of relics of an originally more widespread distribution.

### 5.3 Land reclamation

The value of *Hippophaë* in colonizing poor soils is well-known. It can be put to practical use in helping to stabilize shifting coastal sands and to protecting arable land from wind blown dunes. During the last twenty years there has been a growing interest in the reclamation of land from the effects of industrial dereliction and here too *Hippophaë* can be useful.

In the early stages the problems are purely those of civil engineering; the demolition of buildings, clearance of scrap metal, and control of pollution into waterways and lakes. Large holes created by extractive industries can be filled with industrial waste and urban refuse. The spoil heaps from mining can be landscaped and planted. By the sixties the general principles of land reclamation had been systematised and widely publicised. (GOODMAN 1965, OXENHAM 1966).

Engineers had recognised that planting trees over refuse-filled holes has real practical advantages. The evapotranspiration of the trees reduces the internal water volume within the hole. This helps to produce more aerobic conditions inside the refuse and promotes bacterial action in breaking down the constituents (MOLTZ and BROWNING 1977). Eventually mature woodland could be expected to cover the landfill.

The penetration of tree roots was discovered to have a beneficial effect in raising the oxygen levels inside the refuse particularly if drains and gas vents had been built into the landfill from the outset. (BROWNING and MOLTZ 1978).

Agronomists were called in to advise on suitable trees to plant on dumps and landfills. One of the main problems is the absence of a proper topsoil in which plants can flourish. Only self-sufficient and hardy species are suitable in the earliest stages of reclamation. In the absence of sufficient soil nitrogen the trees and shrubs with nitrogen-fixing nodules on their roots are at a distinct advantage. In the United States the bristly locust (*Robinia hispida* L.) thorny eleagnus (*Eleagnus pungens* L.) and the slash pine (*Pinus elliotii* Engelm.) are popular for primary colonization. The slash pine appeared to grow best, although the other two are nitrogen-fixing species. This is possible because conditions were too acid for the nitrogen-fixers, which seems likely under wet anaerobic conditions (BROWNING and MOLTZ 1978).

The importance of suitable pH for nitrogen-fixers has been recognised when planting these species as colonizers of distressed soils. (CARPENTER and HENSLEY 1979) When lime is added in sufficient quantities to bring the soil pH up to 6 or more nitrogen-fixers like *Eleagnus umbellata*, *E. angustifolium*, *Robinia fertilis*, *R. pseudoacacia*, and *Shepherdia argentea* grew successfully on the distressed soils of strip-mining areas and the margins of new highway constructions.

The sea-buckthorn (*Hippophaë rhamnoides* L.) is a close relative of the *Eleagnus* species mentioned above, and like them is suitable for reclamation work in its native Europe and Asia. In Britain it has been used for the colonization of pulverised fuel ash or waste clays. (RANWELL 1972 a p. 9). It has been recommended for widespread planting on waste

dumps in the Soviet Union as a result of a series of studies at the Voroshilovgrad Forestry Institute. (KELEBERDA et al 1978). Hippophaë was found to improve the soil fertility sufficiently to promote the development of stable mixed woodland over the dumps.

The end result of this process would be the extinction of Hippophaë within the woodland as it cannot tolerate a heavy shade. However its value as a primary colonizer cannot be overstated.





#### 5.4 Hippophaë as a fruit crop

While we are trying to eradicate or at least control the spread of Hippophaë in these islands further east in the Soviet Union and the countries of eastern Europe, and central Asia the plant is actively cultivated as a crop. This may have arisen from an eastern peasant tradition of using the berries as food. The potentiality of Hippophaë as an agricultural species has been neglected in the west because there are fewer suitable locations for it to be cultivated. Most land would usually support a more useful crop of hay or cereals. Central Asia has areas of impoverished soils where Hippophaë is a realistic crop. Many of the great Russian rivers such as the Volga or Don, flowing through normal arable lands, have wide sandy banks and these are used for the cultivation of Hippophaë where no other crop would be practicable (VASCHENKO 1973).

In other areas of impoverished soils such as the desert steppe where most arable crops could not survive, Hippophaë will flourish and can be extended from its natural distributions by planting. The northern taiga by contrast comprises mostly coniferous forest, and is not easy to cultivate once the forest is cleared, due probably to the very short summer growing season as much as anything else. Hippophaë is regularly cultivated along the banks of the Ob in western Siberia. In 1965 the annual crop of its berries was estimated at 100 tons by Dianov. (ROUSI 1971).

In his definitive study of the taxonomy of Hippophaë, Arne Rousi made a digression to present a review of the literature on the cultivation of the plant as a crop. Much of it remains in Russian and untranslated. He mentions the economic

use of berries in jam-making as part of a peasant tradition in Siberia and Finland. This continuing tradition has expanded during the twentieth century into a programme of research and agronomy.

The berries contain high levels of Vitamin C as noted by Darmer (1952) and also a range of carotenoids according to Suomalainen (1947) and Schantz (1952). This latter fact might have been suspected from the bright orange colour of the ripe berries. Cordials made from Hippophaë berries would provide a useful addition to the winter diet in regions like Siberia and Finland with a long winter and a shortage of locally-grown fresh vegetables. Rousi includes a curious record of Hippophaë cultivation in the Hindu Kush at an altitude of 3,900 metres, observed by Vavilov and Bukunin (1929). The plant flowered but set no fruit so it is difficult to see why it was cultivated. It may have been for a windbreak. It was used for this purpose in the Swiss Alps as recorded by Gams (1943) and Darmer (1952) to protect other plants.

The most interesting development recorded by Rousi was the possibility of cultivating, by selecting suitable strains and breeding the variety for its berries. This was mooted by Darmer (1947a, 1952) and Heinisch (1952) two German workers. By 1962 a programme had begun in Russia to select and breed from high-yielding wild strains, and reports emerged from Vasiltsenko and Korolkov (1962) Trofimov (1967) and Kaar (1969).

Since the time of Arne Roussi's review in 1971 the breeding programme has continued and the Russians have investigated

the most efficient methods of propagation of seedlings using sawdust, and mineral substrates together with a variety of dressings (HARANOVICH and ANTANYUK 1979). Techniques have been evolved for propagating small green shoots as cuttings instead of seed (POTAPOV 1978). This would be important when genetic consistency is important to produce high-yielding varieties.

Intensive cultivation of softwood cuttings (young shoots) under artificial mist, using plant growth regulators have produced high yields of suitable plants (TARASENKO et al 1979). There is a long standing research programme at the Timiryazev Academy's Experimental Fruit Growing Station and the state farm Pamyatj Iljicha. This is intended for the northerly non-chernozem zone where fruit production is smaller than in the more southerly parts of the USSR.

This Russian interest in Hippophaë cultivation is indicative of a growing trend in agricultural research in response to demands to increase the world's food supply. Solutions may not lie in trying to find hardy strains of conventional fruit and vegetables capable of growing in arid climates, but rather in selecting natural halophytes and xerophytes of desert habitats to produce varieties which yield fruits or food stores in economic amounts. Success may be achieved not by trying "to make the desert blossom as a rose, but to cultivate the prickly pear" (HOLDGATE and BEAMENT 1977). The great advantage of this approach is that it creates crops which are likely to be familiar to the local subsistence farmer and which can be husbanded with the minimum of expensive modern technology.

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Alan L.F. Mason

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